THE ROLES OF FIVE SPECIES OF *PHYTOPHTHORA* IN INFECTION AND DISEASE OF ROOTS, STEMS, AND PODS OF *THEOBROMA CACAO* L.

by

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This work is dedicated to my very special friends:
my father, John Herbert Newman (in memory), and
my husband, Emanoel Acilino Teotonio da Luz.

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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Phytophthora capsici, P. citrophthora, P. heveae, P. megakarya, and P. palmivora were pathogenic to detached pods, leaves, excised roots of mature trees, and stems of seedlings. Phytophthora citrophthora was generally the most virulent of the species tested on all parts of the cacao plants. Phytophthora palmivora was also highly virulent, and in some tests with stems or leaves it caused lesions as large as those caused by P. citrophthora. Phytophthora capsici and P. heveae were considered moderate pathogens of cacao, but P. capsici was as damaging as P. citrophthora and P. palmivora to leaves. Phytophthora megakarya was the least virulent of the species tested.

Infection occurred in roots of both susceptible and tolerant cultivars inoculated with zoospores, chlamydospores,

or oospores; levels of infection and severity of disease varied according to the species of Phytophthora, the inoculum concentration, and the cultivar tested. Disease severity, numbers of infections, and root weight losses generally were greatest at the highest inoculum levels. Mortality of seedlings was frequent only with P. citrophthora and P. palmivora at 10^6 zoospores per plant or 250 chlamydospores per gram of soil. These two species caused more damage than the other species to root systems of cacao seedlings. Phytophthora megakarya caused little damage and no mortality even at the highest concentrations of inoculum. Periodic flooding of infested soil had little effect on disease in plants growing in soil infested with zoospores or chlamydospores of all species, but flooding increased the severity of disease caused by oospores of P. capsici and P. heveae.

Cultivars PA 30, PA 121, EEG 8 and BE 5 were highly tolerant in zoospore tests with all Phytophthora spp.; their root systems were infected but sustained little or no damage. Cultivars CA 3, PA 169, SIC 806, CAS 2, MA 15, and EET 59 were highly tolerant to P. capsici, P. heveae, and P. megakarya, but had intermediate tolerance to P. citrophthora or P. palmivora. Populations of the Phytophthora spp. were usually highest around roots of the least tolerant cultivars, and in some cases the populations of several species were greatly restricted by highly tolerant cultivars.

CHAPTER 1
INTRODUCTION

Theobroma cacao L., the cacao tree, belongs to the family Sterculiaceae and is native to the rain forests of tropical America. From the headwaters of the Amazon river in the geographically vast Amazon basin region of South America, which is believed to be the center of origin of cacao and related genera, the cacao plant spread mostly in two directions. The 'Forastero' or Amelonado cultivars originated in the eastern part of the region and spread along the Amazon valley, whereas the 'Criollo' cultivars originated in the northern and western basin and spread through the Andes mountains, Central America and southern Mexico (32, 33, 156). According to Cuatrecasas (34), these two forms have sufficiently consistent characters to be recognized as the subspecies T. cacao subsp. cacao and T. cacao subsp. sphaerocarpum. Over 95% of the current world production of cacao comes from vigorous trees of 'Forastero' origin (6). The 'Forastero' cultivars are traditionally grown in Brazil and the West African countries.

Cacao is a small tree, reaching an average height of 5 or 6 meters and having a very characteristic mode of branching; three to five main branches develop from a "fan"

or "jorquette" that forms in seedlings about 1.5 m above the soil surface. The tree attains its full development when about 10 years old; however, the first flowers may be produced when it is about 2 years old. Flowers of the cacao tree are produced in cushions, and pods are formed mainly on the trunk and main branches of the canopy. Because flower anatomy greatly restricts self-pollination, pollination of cacao is essentially entomophilous. Midges belonging to several genera of the family Ceratopogonidea are the most important pollinating insects. Self-pollination is possible, but many cacao cultivars are self-incompatible. This incompatibility, which is unique because the incompatability reaction takes place in the embryo sac, results in the great genetic heterogeneity of cacao and makes breeding programs difficult (16, 139, 155). Of the many thousands of flowers formed on each tree, generally only 1-5% are successfully pollinated and produce pods that mature (117). Fruits of different stages of development may occur on the tree at the same time.

The root system of the mature cacao tree consists of a 50- to 120-cm-long tap root with an extensive system of lateral feeder roots, which grow largely in the top 30 cm of soil. At the tips of the main lateral roots are branches of fine rootlets. The lateral roots grow to lengths of 3 to 5 meters and form an intricately woven mat (22, 95).

Cacao is one of the most important crops in the State of Bahia, Brazil. About 93% of the Brazilian crop is produced in Bahia. Since 1983, Brazil has been the second largest producer of cacao in the world, and in 1985 it was responsible for 22% of the total world cacao production. The 750,000 hectares planted with cacao in Brazil yield an estimated average of approximately 370,000 metric tons of cacao beans annually (33). According to Medeiros (97) and Figueiredo and Lellis (48), one disease, black pod or Phytophthora pod rot, is estimated to cause more than 30% loss of the annual Brazilian production of cacao. Black pod is the most important disease of cacao on a world-wide basis. Although there are some cacao diseases of restricted occurrence which may be more severe and cause greater losses locally, black pod rot is responsible for more crop loss throughout the world than any other disease of cacao (76).

Until recently the causal agent of black pod was considered to be Phytophthora palmivora (Butler) Butler. However, during the past 10 years the taxonomy of the fungi causing black pod and canker of cacao has changed greatly. During the Cacao Phytophthora Workshop held in Harpenden, England, in 1976, it was recognized that instead of a single species responsible for the disease of cacao, three morphological forms (MF1, MF3 and MF4) were apparently involved in what was designated the P. palmivora complex (55). In 1979, Brasier and Griffin (20) published a detailed study of the

taxonomy of the species of Phytophthora affecting cacao. They proposed MF1 as P. palmivora 'sensu Butler' and described MF3 as P. megakarya Brasier and Griffin, a new species. The species designated as MF4 at the workshop, which was considered to be closely related to P. capsici Leonian (65, 73, 141), was later described by several researchers as P. capsici (4, 25, 164). In 1980, a fourth species, P. citrophthora (Smith and Smith) Leonian, was described on cacao from Brazil (25, 68).

According to Brasier and Griffin (20), Erwin (46), and Zentmyer (160), the following characters distinguish P. palmivora, P. megakarya, and P. capsici: sporangium pedicel length, number of chromosomes, colony morphology, presence or absence of chlamydospores, sporangial morphology, and the sporangial arrangement. Phytophthora palmivora has 9 to 12 small chromosomes; stellated and striated colonies; papillate sporangia, varying from near-spherical to ovate-elongate, arranged on a sympodium; and a short pedicel (2-5μ). Phytophthora palmivora is distributed world-wide, although it may not be the predominant species on cacao wherever it occurs. Phytophthora capsici has 9 to 12 small chromosomes; colonies with considerable aerial mycelium and floral patterns; sporangia that are extremely variable, nearly spherical to broadly ovoid or elongated, and have an umbellate formation; and an elongated sporangial pedicel (30-200μ). Phytophthora capsici has been reported in

Central and South America, the West Indies and Cameroon. Phytophthora megakarya, which is the major pathogen in Nigeria and occurs also in other countries of West Africa, has 5 to 6 large chromosomes; colonies with a cottonwool-like mycelium forming a floral, camellioid, or lobel pattern; sporangia nearly spherical to ovate-elongate; and a pedicel of medium length (10-30μ). Phytophthora citrophthora is readily separated from the other three species because it has persistent sporangia, which are often intercalary and vary in size and shape; it forms cottony colonies with floral patterns resembling those of P. capsici, but with abundant chlamydospores. The cacao isolates of P. citrophthora do not form oospores.

Two other Phytophthora spp., P. megasperma Drechsler and P. heveae Thompson, were also reported in association with infected cacao pods under natural conditions. Phytophthora megasperma was reported from Venezuela by Reys et al. (119); it is homothallic, with persistent, inconspicuously papillate sporangia. Phytophthora heveae was first reported occurring occasionally on pods in Malaysia (9, 144); later it was found on cacao in Mexico and Brazil (84, 87). Abundant oogonia are formed homothallically, often in close groups, by P. heveae in culture. The sporangia are caducous and resemble in size and shape those of P. palmivora.

Four of the six species, P. palmivora, P. capsici, P. citrophthora, and P. heveae, have been reported in Brazil (24, 25, 87). Phytophthora citrophthora has been reported as the most virulent species to unwounded, detached and attached pods, stems, and seeds of cacao (28, 80). However, population surveys in Bahia and Espirito Santo, Brazil showed that P. capsici was the predominant species, occurring in 95% of the samples collected in different fields (25).

The occurrence of these various species in different cacao-growing areas of the world has considerable implications for the control of the disease, whether by chemical means, cultural practices, or by the use of resistant cultivars (Lawrence, unpublished). Difficulties in control arise because the Phytophthora spp. from cacao may differ in their abilities to survive and sporulate on different natural substrates (21, 97, 148); in the manner and rate of spread within cacao plantations (90, 97); in their frequencies of occurrence and relative virulences on different parts of the cacao tree (21, 81); and in their tolerances to copper fungicides (27, 49).

The major economic loss caused by black pod disease is diseased pods. Pods can be infected at any stage of their development and at any point on their surfaces. The black pod pathogens can also infect flower cushions, leaves, shoots, trunks, roots and seedlings (75). All of the

infected parts of the tree can give rise to inocula that can cause new infections. The relative importance of these different sources of inoculum on disease development may vary from region to region according to the prevailing Phytophthora spp., cultivars planted, and local climatic patterns. Identification of inoculum sources, their relative importances in each cacao growing region, and knowledge of the manner in which the pathogens are disseminated are of fundamental importance in providing a rational basis for disease control.

Besides the different infected parts of the tree, the upper layers of the soil and surface debris near cacao trees have been considered among the main natural reservoirs of inoculum from which black pod epidemics develop (1, 56, 97, 111). Experiments in Nigeria indicated that infections of nearly 40% of diseased pods were initiated from inoculum in the soil, either by way of rain splash or by ant tents (90). This figure rose to 60% after an interpretation by Gregory et al. (54) showed that a large proportion of pod infections which had "no obvious source" also resulted from soil or litter splashed onto pods by rain (54).

It is uncertain how long and in what form Phytophthora spp. survive in soil. In Ghana, P. palmivora was found to survive the dry seasons in all cacao soils tested (152). Similarly, in Brazil and Jamaica the fungus was recovered throughout the year from soil in areas with high and low

incidences of disease (60, 97). Ward and Griffin (148) reported that P. megakarya survived for at least 18 months and P. palmivora for up to 10 months in buried fiberglass impregnated with both species. Seasonal fluctuations in soil populations of Phytophthora spp. were observed in Nigeria, Brazil, and Jamaica (60, 97, 148). In Nigeria, Ward and Griffin (148) considered the fluctuations of soil populations of P. megakarya to be associated with soil moisture levels, but largely independent of the progress of the aerial phase of the disease during epidemics.

Phytophthora spp. may survive in the soil in various forms. Dormant resting spores are probably most important for survival during the dry season (111). Chlamydospores of P. palmivora were found to remain viable in soil for at least 9 months in Costa Rica (108). In experiments with artificially infested soil, chlamydospores of P. palmivora and P. citrophthora and oospores of P. capsici showed similar survival capacities after 6, 7 and 9 months, respectively (69). More propagules were recovered from soil kept at 18 C than at 24 C, and all three species survived better at a moisture level of -0.8 bar than at -0.2 bar. Turner (143) infested soil with a suspension of P. palmivora zoospores and showed that the inoculum remained viable in soil of intermediate water content for at least 18 months.

Phytophthora megakarya and P. palmivora were isolated from cacao feeder roots of both young and mature trees

during the wet season in Nigeria (148). This suggests, according to Gregory et al. (54), that these two species have a parasitic phase in the soil and gives indications that Phytophthora spp., particularly P. megakarya, may be much more important in the infection and disease of roots than previously thought. However, the extent to which root infection by any of the Phytophthora spp. occurs and the economic importance of root disease are still not known.

Lass (75) suggested that detailed work on root infection of mature trees needs to be carried out in order to understand better the role of root infection in the disease cycle. Gregory (53) listed three possible ways that root infection could be harmful in cacao plantations: 1) providing a reservoir of inoculum for pod infections, 2) leading to tree debility and a decline in yields in many areas, or 3) resulting in diseases of the roots of young trees on replanting and causing growth stunting. Consequently, a detailed study of the roles of infested soil and infected roots in the epidemiology of Phytophthora diseases of cacao needs to be conducted to elucidate the ability of the various Phytophthora spp. to infect cacao roots and produce inoculum that may function in the subsequent development of black pod and other forms of disease. This information is needed for the development of improved biological and cultural methods of disease control and for the selection of resistant cultivars (161).

The selection of cultivars with resistance or tolerance to the different species occurring in each region of the world is of primary importance for the management of the disease. The terms resistance and tolerance used in this study are defined as by Agrios (3). Resistance is the ability of a host to overcome, completely or in some degree, the effect of a pathogen or other damaging factor. Tolerance is the ability of a plant to sustain infection or the effects of a disease without dying or suffering serious injury or crop loss. Since Phytophthora spp. differ in virulence on pods or other organs (20, 26, 80, 81), it is most important to test resistance in pods to the various species. According to Lawrence and Luz (80), pods of cultivars may not necessarily present the same resistant or susceptible reaction to all of the Phytophthora spp. present in a given area. For example, in Bahia, Brazil, the cultivar 'Catongo' is moderately resistant to P. capsici, but susceptible to P. palmivora and P. citrophthora; Scavina 6 and EET 59 are resistant to P. capsici and P. palmivora, but susceptible to P. citrophthora (80, 81). The development of a screening test for root resistance may have importance in controlling the potential primary source of inoculum.

Cultivars of cacao that are immune to the black pod disease have not been found; however, there are varying degrees of tolerance or resistance (80, 87). Cultivars with consistently smaller mean lesion diameters on pods after

inoculation are considered more resistant than cultivars whose pods had larger lesion diameters in all of the tests performed under uniform conditions. A grading system or index was proposed by Luz and Yamada (86) to measure and compare the reactions of cultivars to pod inoculations with Phytophthora spp. The resistance or susceptibility of cultivars in their system was based on inoculation tests conducted under field conditions during three consecutive years. Each year at least 10 attached pods of all tested cultivars were each inoculated on two diametrically opposite points with P. capsici, P. citrophthora or P. palmivora. Based on rankings similar to those used to evaluate results of stem inoculations (76, 80, 81, 165), areas of lesions were used to grade the resistance or susceptibility of cultivars to pod inoculations. The index values varied for each species according to its virulence to the cacao plant. The mean diameters of the lesions caused on pods by P. capsici were one half and one third of those caused by P. palmivora and P. citrophthora, respectively. Cultivars CA 3, PA 121, and SCA 12 showed resistance to P. capsici and P. palmivora but not to P. citrophthora; PA 30 and PA 169 were considered resistant to P. capsici and P. palmivora and tolerant to P. citrophthora. The overall objectives of this research were to investigate 1) the comparative roles of P. capsici, P. citrophthora, P. heveae, P.megakarya, and P. palmivora in root disease, stem lesions, leaf lesions, and pod rot of

cacao plants; 2) the relationship of inoculum density in soil to incidence of disease in cacao roots; and 3) disease resistance or tolerance in selected cacao cultivars in relation to forms and densities of inoculum of Phytophthora spp.

CHAPTER 2
COMPARISONS OF THE ROLES OF *PHYTOPHTHORA CAPSICI*, *P. PALMIVORA*, *P. CITROPHTHORA*, *P. MEGAKARYA* AND *P. HEVEAE* IN ROOT DISEASE, STEM LESIONS, LEAF LESIONS, AND POD ROT OF CACAO PLANTS.

Introduction

Many parts of the cacao plant can be infected by *Phytophthora* spp., including flower cushions (21, 35, 37, 62, 91, 107), flowers (62), leaves (62, 91, 109, 112, 147), peduncles (96, 136), shoots (18), trunks (82, 90, 126, 137), roots (35, 112, 145, 148), seeds (18), and seedlings (25, 30; Luz, unpublished). However, almost all of these reports were made before species other than *Phytophthora palmivora* were recognized as pathogens that cause black pod. Since the recognition of several different *Phytophthora* spp. as black pod pathogens, attempts have not been made to determine the precise contribution of each species as a pathogen of different parts of cacao trees.

In addition to *P. palmivora*, *P. capsici*, *P. citrophthora*, and *P. megakarya*, several other *Phytophthora* spp. have been isolated and shown to cause cacao pod rot. *Phytophthora megasperma* has been reported only in Venezuela (119), and *P. heveae* Thompson has been isolated and shown to cause black pod on cacao in Malaysia (9, 144), and Mexico

(84). Recently P. heveae was isolated from cacao roots in Brazil (87).

In the last few years the diversity of Phytophthora spp. causing black pod in different parts of the world has been studied intensively. As a result of this work, it is now known that different combinations of species of Phytophthora often occur in each country where cacao is grown (Table 2.1). In Central and South America, P. capsici generally predominates, although it occurs together with P. palmivora in several countries. Exceptions are found in Colombia, Costa Rica, the Dominican Republic, and Panama, where only P. palmivora has been reported to date. Phytophthora capsici, P. citrophthora, P. heveae, and P. palmivora all cause disease in Brazil and Mexico. Phytophthora palmivora and P. megakarya occur in most of the African countries where cacao is an important crop. However, P. megakarya is the most damaging pathogen in Africa. Other species have been reported recently in some African countries; for example, P. capsici and P. citrophthora have been found in Cameroon and Nigeria (12) and in Cameroon (Bakala, unpublished), respectively. In Asia, P. palmivora predominates, but P. heveae is also considered a cacao pathogen in Malaysia and the Solomon Islands (9, 144).

In Brazil, P. palmivora was isolated from pods, chupons, leaves, flowers, flower cushions and cankers (25).

Table 2-1. Geographical distribution of the Phytophthora spp. inciting diseases of cacao.

Countries	Species of Phytophthora[y]					
	Pal	Cap	Megak	Citro	Hev	Megasp
Western Hemisphere						
Brazil	+[z]	+		+	+	
Colombia	+					
Costa Rica	+					
Dominican Republic	+					
El Salvador		+				
Ecuador		+				
Guatemala		+				
Honduras		+				
Jamaica	+	+				
Mexico	+	+		+	+	
Panama	+					
Trinidad	+	+				
Tobago	+	+				
Venezuela	+	+				+
West Africa						
Angola	+					
Cameroon	+	+	+	+		
Equatorial Guinea			+			
Fernando Po			+			
Gabon	+		+			
Ghana	+		+			
Ivory Coast	+		(+)?			
Nigeria	+	+	+			
Republic of Congo	+		(+)?			
Sierra Leone	+					
Sao Tome	(+)?		+			
Togo	+		+			
Zaire	+					
Asia and Oceania						
Fiji	+					
India	+					
Malaysia	+		(+)?		+	
Papua New Guinea	+					
Solomon Islands	+				+	
Sri Lanka	+					

[y]Pal = P. palmivora; Cap = P. capsici; Megak = P. megakarya; Citro = P. citrophthora; Hev = P. heveae; Megasp = P. megasperma. Occurrences based on reports by Brasier et al. (21); Chandra-Mohanan (29); Del Carmen (38); Erselius and Shaw (45);

Table 2-1 Continued.

Fagan(47); Lass (75); Lawrence (unpublished); Lozano and Romero (84); Luterbacher, unpublished; ; Luz et al. (87); Montes B. (105); Zentmyer (162).

[z] + =species of Phytophthora confirmed on cacao in this country; (+)? = unsubstantiated report.

It is often associated with pre- and post-emergence damping off of young cacao seedlings or rootstocks (25; Luz, unpublished). Phytophthora palmivora also causes die-back of young and old cacao trees during the dry season of the year in poorly shaded areas. Phytophthora capsici was isolated mainly from pods, but also from chupons and leaves, whereas isolates of P. citrophthora were obtained only from pods and trunks of cankerous trees (25).

It is probable that Phytophthora spp. have adapted their methods of attacking cacao and surviving in the host, soil, and non-host plants to each of the areas in which this disease is found. In this context, little is known about the four principal species of Phytophthora that cause black pod, and, therefore, studies on the abilities of the different species to cause disease of various organs of cacao are important. Since P. heveae was also isolated recently in Brazil, it was included in some of the studies in order to determine its potential as a cacao pathogen.

The objectives of this study were to evaluate the roles of P. capsici, P. citrophthora, P. heveae, P. megakarya and P. palmivora in root infection, stem lesions, leaf blight, and pod rot of cacao plants in Itabuna, Bahia, Brazil and at the University of Florida in Gainesville.

Materials and Methods

Isolates P26 of P. palmivora, P23 of P. capsici, P62 of P. citrophthora, Etoun of P. megakarya, and P208 of P. heveae were used in all of the comparative tests performed in this study. The first three isolates were obtained from naturally infected pods in Itabuna, Bahia, Brazil and have been used before in tests to compare the virulence of these Phytophthora spp. in Brazil, and in screening tests for disease resistance that have been performed at CEPLAC in Itabuna, Brazil (26, 80, 81, 86, 129). The isolate Etoun of P. megakarya originated from Cameroon and was supplied by Mr. J. Bakala of the Institut de Recherches Agronomique de Nkolbisson, Yaounde, Cameroon. Isolate P208 of P. heveae was obtained from naturally infected roots of cacao collected at Urucuca, Bahia, Brazil (87).

Zoospores of P. palmivora, P. capsici, P. megakarya, and P. heveae were produced by seeding petri plates containing 15 ml of carrot-agar (CA) (200 g of carrot decoction, 17 g of agar and water to 1000 ml) with two 4-mm plugs from the margin of a 2-day-old culture on CA or corn meal agar (CMA). All cultures, except those of P. megakarya, were incubated in an incubator at 27 C for 10 days under continuous fluorescent white light (two General Electric F40D daylight fluorescent lamps, 1,200 w/cm^2); cultures of P. megakarya were maintained for 5 days in

darkness and then an additional 5 days in continuous light. After 10 days, each culture was suspended in 15 ml of water. Zoospores were released from sporangia after 20 minutes at 5-8 C and an additional 25 minutes at room temperature. Small samples of the zoospore suspensions were agitated in test tubes for 60 seconds on a Vortex mixer to induce motile zoospores to encyst. Concentrations of zoospores were determined by counting five fields for each of 10 samples from each of the tubes on a standard hemacytometer.

Since most sporangia of P. citrophthora are embedded in solid media, zoospores of this species were produced in petri plates containing 15 ml of carrot-extract broth (200 g of carrot decoction in 500 ml of water to make 200 ml of carrot extract; water added to bring to 1000 ml) using the simplified version of the method of Tsao and Oster (142) as described by Lawrence and Luz (79). After 9 days of incubation at 27 C under continuous light, the medium was drained from the plates and the mycelia were washed four times with 20-ml aliquots of an autoclaved solution of 10^{-4} M 2-(n-morpholino)-ethane sulfonic acid (MES) in deionized water; the solution was adjusted to pH 6.2 with 1 N KOH (102). After 24 hours under light the drained cultures were suspended in 10 ml of MES. Motile zoospores were produced as described above.

Full sized but non-matured pods of the cacao cultivar 'Comum,' the predominant cultivar in commercial areas near

CEPLAC, were harvested from the field immediately prior to use and washed in tap water. Pods were randomly placed in wooden boxes (90 cm long, 35 cm wide, and 20 cm high), which were lined and sealed with transparent polyethylene sheeting and contained a reservoir of water. Pods (usually 10 per box) did not contact the water in the box, or each other. Ten replicate pods were inoculated with each species using the procedure modified by Lawrence (78). Each pod was inoculated at each of two points on its surface with 10^3 zoospores in a 0.01-ml water drop. The inoculum droplets were placed in a furrow of the pod in the middle of the peduncular and apical hemispheres. Immediately after inoculation the boxes were sealed and incubated under laboratory conditions (temperatures of 25-28 C). Three replicated tests were done on August 8, August 23, and November 5, 1988. Five days after inoculation the diameters of the lesions were measured in two directions at right angles to each other, parallel to the transverse and longitudinal axes of the pod; lesion diameter equalled the mean of the two values. The percentage of infection was also estimated by dividing the number of lesions obtained on pods of each cultivar in relation to the total number of inoculated points. The data were analyzed as a completely randomized block design in which each block contained two pods inoculated with each species.

For stem inoculations seedlings of three cultivars, SIC 19 (a 'Comum' selection), SIC 864 (a 'Catongo' selection; 'Catongo' is a natural mutation for white flowers of the cultivar 'Comum'), and PA 121 (a selection from Parinari, Peru), were grown in pasteurized soil under greenhouse conditions for 6 months. The soil was pasteurized for 4 hours at 80 C on each of 2 consecutive days and maintained for 4 weeks to allow microbial recolonization before use in tests. Seeds of the three cultivars were obtained from natural pollination. In order to have three replications over time, seeds of the same cultivars, when available, were planted at three different dates. The first test consisted of 30 plants of each of two cultivars (SIC 19 and SIC 864) inoculated with each species of Phytophthora tested. Twelve and 30 plants of each of the three cultivars were inoculated with each species in the second and third test, respectively.

Stems of plants were inoculated with a wound method described previously by Zentmyer et al. (165) and modified by Lawrence (78). Each of the replicate cacao seedlings was inoculated with a 4-mm-diameter mycelial disk of a 4-day-old CA culture of one of the five Phytophthora spp., or a CA disk free of fungi (control). After 18 days under greenhouse conditions, the bark around the inoculation site was removed and the margins of the discolored lesions on the stems of plants were traced. Areas of the lesions were

evaluated using the micro-comp data acquisition system developed by Southern Micro Instruments Inc. (120 Interstate North Parkway East, Atlanta, Georgia 30339). Data from each inoculation test were analyzed individually as a two-factor (species and cultivars) factorial in a completely randomized block design. Interactions among species and cultivars were also analyzed.

Young, unhardened leaves, and mature leaves of the cultivar 'Comum' were detached from trees, washed in running tap water, rinsed in deionized water, blotted dry with paper towels, and inoculated with 0.01-ml droplets of zoospore suspensions containing 10^3 zoospores per droplet. Two inoculum drops were placed at different points on the upper leaf surface, and leaves treated with drops of water served as controls. Inoculated leaves were incubated in damp plastic boxes on a wet, sterile, sponge surface to which drops of sterile water were added, when necessary, to maintain high humidity. The boxes were enclosed in plastic bags. Ten leaves were inoculated with each species of Phytophthora. Three days after inoculation, lesion diameters for all of the Phytophthora spp. were measured through the centers of lesions transversely and longitudinally to the mid vein. The percentage of infection was also estimated by dividing the number of lesions by the total number of inoculation points. Tests were performed three times and data from each test were analyzed individ-

ually and the means compared by Duncan's multiple range test ($P<0.01$).

Roots of mature cacao trees were dug from the field, divided into sections approximately 15 cm long, and brought immediately into the laboratory for inoculation. The diameters of the roots varied between 0.5 and 1.0 cm. The roots were washed, surface disinfested with 70% ethanol for 30 seconds, and rinsed twice in sterile, deionized water. Each of ten excised sections of root were inoculated with a 0.01-ml droplet of a zoospore suspension of each of the five species containing the same concentrations used for detached leaves, or water droplets as a control. The root pieces were incubated under the same conditions described for detached leaves for 12 hours before the points of inoculation were marked and the roots were covered with pasteurized soil. The inoculated roots and controls were sampled over time for lesion development as well as for other fungal contamination. Based on preliminary tests, the final evaluations were made 5 days after inoculation by removing the bark around the inoculation sites and measuring the longitudinal and transverse growth of the discolored lesions. Pieces of tissue from the margins of the lesions were also plated on a medium selective for Phytophthora spp. (63). The medium contained 10 mg of pimaricin, 250 mg of ampicillin, 10 mg of rifampicin, 100 mg of pentachloronitrobenzene, 50 mg of hymexazol, and 17 g of cornmeal agar in

1.0 L of deionized water. The test was repeated one time. The data were analyzed as a completely randomized block design, in which each box containing one excised section of root inoculated with each species was considered as a block.

Results

All of the Phytophthora spp. infected non-wounded, detached pods of cacao to some degree in each of the three replicated tests (Table 2-2). Differences were evident in the sizes of lesions caused by the five species of Phytophthora tested (Figure. 2.1). Phytophthora citrophthora caused the largest lesions and P. megakarya caused the smallest lesions. Lesions of intermediate sizes were caused by P. palmivora, P. capsici, and P. heveae, but lesions caused by P. palmivora were larger than those of the latter two species. The results of the three tests performed were similar; however, the sizes of the lesions caused by P. capsici, P. heveae, and P. megakarya were greater in the first two tests than in the last test ($P<0.01$). Not all of the inoculated points were infected when P. capsici, P. heveae or P. megakarya were used as inoculum. The highest number of non-infected inoculated sites generally occurred with P. megakarya.

Cankers formed on wounded stems of seedlings of the three cacao cultivars after inoculation with each of the

Table 2-2. Average lesion diameters and percentages of infection on detached, nonwounded pods of 'Comum' cacao 5 days after inoculation with a drop of zoospore suspension of one of five Phytophthora spp.

Species[x]	Average Lesion Diameter[y] (cm)			Infection[z] (%)		
	test 1	test 2	test 3	test 1	test 2	test 3
P. citrophthora	12.33a	9.86a	9.79a	100	100	100
P. palmivora	8.38b	9.46a	8.16b	100	100	100
P. heveae	4.92c	4.53b	5.86c	85	90	90
P. capsici	4.19c	3.24b	6.11c	80	75	100
P. megakarya	1.58d	1.47c	4.26d	65	80	90

[x]Zoospore suspensions of each species were calibrated to have 10^3 zoospores per 0.01-ml droplet; one droplet was used per inoculation point.

[y]Expressed as the average diameter of lesions from 20 points inoculated with each species per test; means followed by the same letter in each column were not significantly different according to Duncan's multiple range test (P=0.01); mean comparisons were valid among treatments within each test.

[z]Infection, expressed as the percentage of 20 inoculated sites that were infected 5 days after inoculation, was determined by plating a small piece of pod on a selective medium.

Figure 2-1. Lesions of Phytophthora spp. on 'Comum' cacao pods 5 days after inoculation with droplets of zoospore suspensions of P. megakarya (MG); P. capsici (23); P. heveae (DE); P. palmivora (26); and P. citrophthora (62).

Figure 2-2. Lesions of Phytophthora spp. on seedlings of cacao cultivar SIC 864 18 days after stem inoculation with mycelial disks of Phytophthora heveae (DE); P. megakarya (MG); P. capsici (23); P. palmivora (26); and P. citrophthora (62).

Figure 2-3. Average lesion areas (cm^2) in test 1, 18 days after stems of seedlings of the cultivars SIC 19 and SIC 864 were inoculated with disks of mycelium of Phytophthora spp. Bars among each cultivar marked with same letter are not significantly different according to Duncan's multiple range test ($P<0.01$).

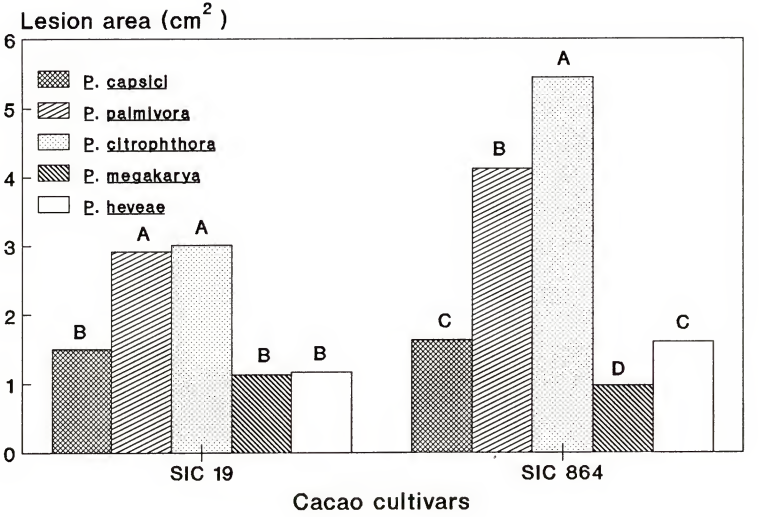

Lesion area (cm^2)
6
5
4
3
2
1
0
P. capsici
P. palmivora
P. citrophthora
P. megakarya
P. heveae
A
A
B
B
B
B
A
C
C
D
SIC 19
SIC 864
Cacao cultivars

Figure 2-4. Average lesion areas (cm^2) in test 2, 18 days after stems of seedlings of the cultivars PA 121, SIC 19, and SIC 864 were inoculated with disks of mycelium of Phytophthora spp. Bars among each cultivar marked with same letter were not significantly different according to Duncan's multiple range test ($P<0.01$).

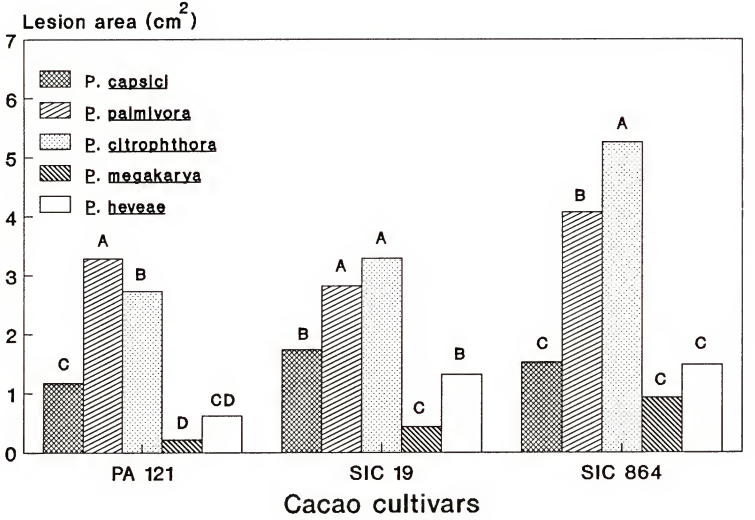
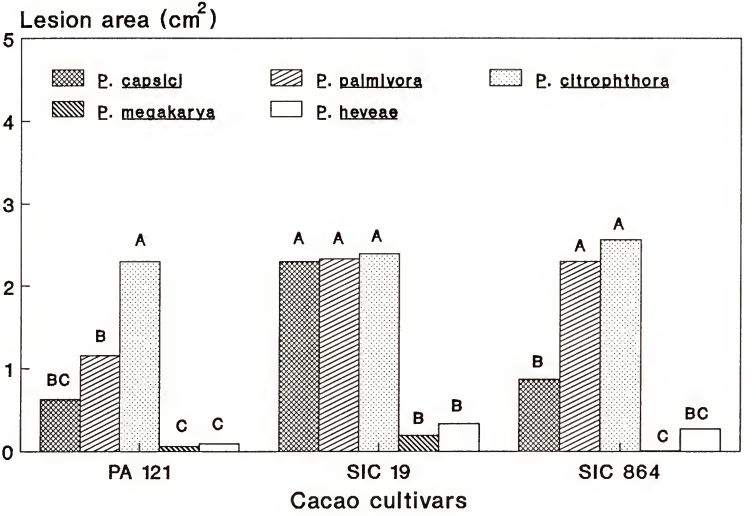

Lesion area (cm²)
7
6
5
4
3
2
1
0
P. capsici
P. palmivora
P. citrophthora
P. megakarya
P. heveae
A
B
C
A
A
B
C
B
A
B
C
C
D
CD
PA 121
SIC 19
SIC 864
Cacao cultivars

Figure 2-5. Average lesion areas (cm^2) in test 3, 18 days after stems of seedlings of the cultivars PA 121, SIC 19, and SIC 864 were inoculated with disks of mycelium of Phytophthora spp. Bars among each cultivar marked with same letter are not significantly different according to Duncan's multiple range test ($P<0.01$).


Lesion area (cm²)
5
4
3
2
1
0
P. capsici
P. palmivora
P. citrophthora
P. megakarya
P. heveae
BC
B
A
C
C
A
A
A
B
B
B
A
A
C
BC
PA 121
SIC 19
SIC 864
Cacao cultivars

five species of Phytophthora (Figures 2.2-2.5). The largest lesions developed from infections by P. citrophthora and P. palmivora, except in test 3 in which lesions caused by P. palmivora on PA 121 were not greater than those caused by P. capsici. Also in test 3, P. capsici caused as large of lesions as P. palmivora and P. citrophthora on SIC 19. Phytophthora heveae caused lesions as large as those caused by P. capsici and P. megakarya on all cultivars in the three tests except on SIC 19 in test 3. Phytophthora citrophthora and P. palmivora killed some plants of cultivars SIC 864 and SIC 19 by completely girdling the seedling stems (Table 2.3). Phytophthora citrophthora also killed 17% of the PA 121 seedlings in the second test.

Few consistent differences in the responses of cultivars were exhibited over the three tests when the effects of all species over cultivars were analyzed. Cultivar SIC 19 had smaller stem lesions than SIC 864 when inoculated with P. citrophthora and P. palmivora in tests 1 and 2, but no differences were detected in test 3 (Figures 2.3-2.5). Unfortunately, seeds of PA 121 were not available for test 1, and the smaller lesions that occurred with P. palmivora on PA 121 than on the other cultivars in the third test were not apparent in the second test. The average lesion diameters caused by all of the species on all of the cultivars were consistently reduced in the third test (Figure 2.5), and only one plant each of SIC 864 and SIC 19 inoculated

Table 2-3. Comparisons of percentages of infection and mortality 18 days after stem inoculations with disks of mycelium of cultures of Phytophthora spp. in 6-month-old seedlings of SIC 19, SIC 864 and PA 121 in three different tests.

Species	Test[w]	Percent Infection[x]			Percent mortality[y]		
		PA 121	SIC 19	SIC 864	PA 121	SIC 19	SIC 864
P. capsici	1	n[z]	100	100	n	0	0
	2	100	100	100	0	0	0
	3	100	100	93	0	0	0
P. citrophthora	1	n	100	100	n	13	13
	2	100	100	100	17	42	25
	3	100	100	100	0	7	7
P. heveae	1	n	100	100	n	0	0
	2	100	100	100	0	0	0
	3	73	73	53	0	0	0
P. megakarya	1	n	100	100	n	0	0
	2	75	93	100	0	0	0
	3	47	47	7	0	0	0
P. palmivora	1	n	100	100	n	7	10
	2	100	100	100	0	8	17
	3	100	100	100	0	0	0

[w]Test 1 was initiated in October, 1988 with 30 plants of each cultivar; Test 2 was initiated in November, 1988 with 12 plants of each cultivar; and test 3 was initiated in February, 1988 with 30 plants of each cultivar.

[x]Infection, expressed as the percentage of 30 plants for tests 1 and 3 and 12 plants for test 2 that were infected 18 days after inoculation, was determined by plating stem tissue on a selective medium.

[y]Mortality=the percentage of 30 plants for tests 1 and 3 and 12 plants for test 2 that died 18 days after inoculation.

[z]n=not tested

with P. citrophthora was killed. Test 3 was performed in February, whereas tests 1 and 2 were conducted in October and November, respectively. All three cultivars tested, however, had only small lesions caused by P. heveae and P. megakarya. The cultivars included in tests 1 and 2 and SIC 864 in test 3 also had smaller lesions caused by P. capsici than by P. citrophthora and P. palmivora.

The lesions caused by Phytophthora spp. on detached, immature leaves, of cacao trees developed within 24 hr after inoculation under the high humidity conditions of the test (Table 2.4). Larger lesions occurred on immature 'Comum' leaves inoculated with P. citrophthora, P. palmivora and P. capsici than on leaves inoculated with P. megakarya in both tests, or with P. heveae in the first test. The percentages of infection obtained with the latter two Phytophthora spp. also were consistently lower than those obtained with P. citrophthora, P. palmivora, and P. capsici ($P<0.01$). Mature leaves are considered to be more resistant than immature leaves, but inoculum drops dried on the mature leaves very rapidly and prevented accurate estimates of disease. Thus, the results of tests with mature leaves are not presented.

Since the averages of areas of lesions on excised roots of mature cacao trees did not differ significantly among the three experiments, the data were combined for comparisons (Table 2.5). The largest lesions developed on roots inoculated with P. citrophthora. Phytophthora palmivora, P.

Table 2-4. Average diameters of lesions and percentages of infection 3 days after inoculation of immature, detached leaves of 'Comum' cacao with zoospores of five Phytophthora spp.

Species[x]	Average Lesion Diameter[y]		Infection[z]	
	Test 1 (cm)	Test 2 (cm)	Test 1 (%)	Test 2 (%)
P. citrophthora	5.13a	4.32a	100	90
P. palmivora	4.37a	4.78a	100	100
P. capsici	3.98a	4.34a	100	90
P. heveae	2.02b	2.98ab	60	70
P. megakarya	1.67b	1.36b	60	40

[x]Points on leaves were inoculated with 10^3 zoospore in a 0.01-ml drop of water.
[y]Expressed as the average diameters of lesions from 20 points inoculated with each species during each test; means followed by the same letter in columns were not significantly different according to Duncan's multiple range test ($P<0.01$). Mean comparisons were valid among treatments within a test.
[z]Infection, expressed as the percentage of 20 inoculated sites that were infected 3 days after inoculation, was determined by plating pieces of leaf tissue on a selective medium.

Table 2-5. Average of lesion diameters and percentages of infection for excised roots of 'Comum' cacao 5 days after inoculation with zoospores of five Phytophthora spp.

Species[x]	Average Lesion Diameter[y] (cm)	Infection[z] (%)
P. citrophthora	3.59a	100
P. palmivora	2.78b	100
P. heveae	2.40c	100
P. capsici	2.24c	100
P. megakarya	0.82d	87

[x]Points on roots were inoculated with 10^3 zoospores in a 0.01-ml drop of water.
[y]Expressed as the average of lesion diameters on 30 root pieces inoculated with each species; means followed by the same letter in columns were not significantly different according to Duncan's multiple range test (P=0.01).
[z]Infection, expressed as the percentage of 30 roots that were infected 5 days after inoculation, was determined by plating root pieces on a selective medium.

capsici, and P. heveae were intermediate in virulence to detached roots, although the lesions caused by P. palmivora were significantly larger in area than those caused by P. heveae and P. capsici. Phytophthora megakarya was less virulent than the other four Phytophthora spp. tested, and only small lesions were formed by this species.

The deterioration of detached roots became apparent in the control roots on the sixth day after inoculation, and no measurements were taken after 6 days. All Phytophthora spp. were reisolated from the infected, detached roots, but not from noninoculated roots. Although a medium selective for Phytophthora spp. was used for root isolation, bacterial contamination was associated with roots in all of the isolation plates.

Discussion

Phytophthora capsici, P. citrophthora, P. heveae, P. megakarya, and P. palmivora are all pathogenic to detached pods, seedling stems, excised roots, and leaves of cacao. However, great differences occurred in the virulence of the five Phytophthora spp. associated with diseases of different parts of the cacao plants tested. This report is believed to be the first to compare the roles of all of these species as pathogens of Theobroma cacao. Previous comparisons of the virulence of Phytophthora spp. to cacao

included: P. capsici and P. palmivora in Brazil and Jamaica, (47, 80); P. palmivora and P. megakarya in Nigeria (20); and P. capsici, P. palmivora and P. citrophthora in Brazil (28, 80). Phytophthora heveae was reported only recently in Mexico and Brazil (84, 87).

Phytophthora citrophthora was generally the most virulent species on all parts of cacao tested. The high virulence of this species was demonstrated previously in Brazil (28, 80). Development of lesions in pods, leaves, and roots and death of stem-inoculated seedlings of susceptible cultivars occurred rapidly under optimum environmental conditions. Wherever it occurs, Phytophthora citrophthora presents a serious threat to cacao. For example, severe black pod observed in a commercial plantation in Buerarema, Bahia, Brazil in 1985 was caused primarily by P. citrophthora (Luz and Silva, unpublished). This species has also been associated with diseases of roots and trunks of citrus (51), walnut trees (100) and almonds (153).

The ability of P. citrophthora to produce abundant chlamydospores enhances its survival in soil or in affected parts of the host. However, the fact that P. citrophthora sporangia are non-deciduous may account for the slow spread of this species on the aerial parts of the cacao tree on which it has been shown to be so highly pathogenic (28, 80). In experiments on cacao pods in Brazil, Luz and Campelo (85) observed that pods infected with P. citrophthora at 25-27 C

developed a cottony mycelium on the surface of the lesions 3 days after inoculations. Although sporangia and chlamydospores were formed, the addition of water did not stimulate zoospore liberation even after 1 hour. Either indirect germination of zoosporangia of this species requires environmental conditions different than those tested, or zoospores are not formed readily on natural substrates.

Phytophthora palmivora was also highly virulent on different parts of the cacao tree. This fungus has been isolated from pods, chupons, flowers, flower cushions, seedlings, trunks, and roots of cacao in the many areas of the world where this widely disseminated species occurs (75). Phytophthora palmivora was more virulent than P. megakarya on detached pods in Nigeria (20) and proved to be more virulent than P. capsici on pods in Jamaica (47). The behavior of P. palmivora and P. capsici in the present study supports the findings of Lawrence et al.(81) and Campelo et al. (25) in Brazil, in which P. palmivora was more virulent than P. capsici but less virulent than P. citrophthora. Only P. palmivora may be isolated from some areas of Bahia, Brazil where black pod symptoms are severe (Luz, unpublished) and sporadic outbreaks of black pod during the "temporao" period (minor cacao harvest) in Brazil also have been associated with attacks of P. palmivora.

Phytophthora capsici has been considered the predominant species in Bahia (25). The prevalence of this

pathogen appears to be associated with its ability to produce abundant sporangia that release zoospores at temperatures around 20 C, which are common in Bahia during the period of "safra" (the main cacao harvest) (85). In inoculations of cacao pods with P. citrophthora, P. palmivora and P. capsici in Brazil, Luz and Campelo (85) observed that only P. capsici sporulated abundantly on the surface of pods incubated at 20 C; thus, the ability of this species to sporulate abundantly over a range of 15-30 C may be important in explaining its prevalence on cacao in Bahia, Brazil. In contrast to its low aggressiveness to other cacao organs, P. capsici was as pathogenic as P. citrophthora and P. palmivora on cacao leaves (Table 2.4). The sporulation of this species on cacao leaves and petioles may be a supplementary source of inoculum for secondary infections occurring inside the cacao tree canopy. Hardened leaves are much less susceptible than unhardened young leaves; however, under conditions of prolonged high humidity in the field, infections may progress along intermediate secondary and main veins of mature leaves, invade the petioles, and cause leaves to drop off (92). Sporulating Phytophthora spp. on leaves on the soil surface may be sources of inoculum for new infections on the same tree or on adjacent ones. Newhook and Jackson (109) isolated P. palmivora from samples of the leaf litter of cacao plantations in the Solomon Islands, and demonstrated that

fallen cacao leaves are potential sources of splash-dispersed inoculum.

Phytophthora megakarya caused little damage to seedling stems, pods, leaves, and roots in experiments in Florida. Perhaps the laboratory and greenhouse environmental conditions under which experiments were conduced were not conducive to disease development by this species. Since P. megakarya is the predominant species in Africa, it would be interesting to conduct similar virulence tests with all of the species known to infect cacao under conditions suitable for disease development with this pathogen. Cameroon appears to be the ideal place to undertake these pathogenicity tests, because P. megakarya, P. palmivora, P. citrophthora, and P. capsici have all been reported there (Bakala, unpublished). Preliminary tests done in Florida with four A^1 isolates of P. megakarya, which originated from Nigeria and were provided by G.A. Zentmyer from the University of California Culture Collection, showed very low virulence when compared to the Brazilian isolates of the other Phytophthora spp. To avoid the possibility of loss of pathogenicity by long term storage, nine fresh isolates of P. megakarya were obtained from Cameroon and compared to the four California isolates by stem inoculations on seedlings of different cacao cultivars in Florida. Just one isolate among the 13 isolates tested was highly virulent to all the cacao cultivars tested. The other isolates gave similar

results to the previous test, and the differences in pathogenicity among them were minimal and insignificant. Subsequent taxonomic examinations of all of the isolates, revealed that the highly virulent isolate (Bou) was really P. citrophthora and all of the other isolates were P. megakarya. The Cameroon isolate, Bou, was as virulent as P-62, the Brazilian isolate of P. citrophthora, to seedlings of cacao in stem inoculations for comparisons of virulence.

The predominance of P. megakarya in Africa and P. capsici in Brazil is probably related more to their adaptations to environmental conditions or ecological interactions than to their higher pathogenicity to cacao. Brasier, et al. (21) stated that, although the measurements of size of lesions on wound-inoculated pods showed that P. palmivora lesions developed considerably faster than the lesions caused by P. megakarya, under Nigerian field conditions P. megakarya established initial infections of pods more efficiently than P. palmivora (20). In Nigeria, P. palmivora is able to survive the dry season in infected cushions; conversely, P. megakarya seldom becomes systemic in flower cushions, and it rarely survives the dry season in this manner (21). Results presented here support the conclusion that P. megakarya was the least virulent to cacao among the five Phytophthora spp. evaluated.

Lesions caused by P. heveae on pods, leaves, and detached roots were not significantly different from lesions

caused by P. capsici (Tables 2.2, 2.4, and 2.5). These two species may be considered moderate or poor pathogens to cacao when compared with the other species tested; neither species caused extensive lesions.

The findings from the present study support the observations of Turner (144) that P. heveae causes only slight damage to cacao. However, in contrast to Turner's (144) report, P. heveae penetrated non-damaged cacao pods in the present study. Phytophthora heveae caused lesions on non-wounded pods, leaves, and excised roots of cacao. Inoculated pods incubated under high humidity showed profuse formation of oospores and sporangia at the surfaces of lesions, but chlamydospores were rare. As P. heveae has not been isolated from naturally infected pods in the field in Brazil, it may be more adapted to soil conditions than to aerial conditions. The homothallic production of large numbers of oospores, as well as chlamydospores and sporangia, in infected plant tissue may facilitate its survival under soil conditions, or its function as a root pathogen.

Inoculations did not always result in lesions on pods, leaves, and roots of cacao. Although this occurred on some occasions with all species tested, the numbers of pod and leaf sites that did not develop symptoms were greater with P. capsici, P. heveae, and P. megakarya than with P. citrophthora or P. palmivora (Tables 2.2 and 2.4).

To compensate as much as possible for the high variability existing in cacao populations and since the pods were obtained from open pollinated trees, two points were inoculated on each pod in the present study. Lesions developed from just one inoculation site on some pods or leaves inoculated at two points (Fig. 2.1). In some instances stem inoculations, which require wounding for infection and generally lead to lesion formation, did not result in lesions. In the third test with seedling stem inoculations, 26-93% of the seedlings of the three cultivars inoculated with P. megakarya and P. heveae did not develop lesions. This experiment was conducted under greenhouse conditions in the summer when the range of temperatures was 25-30 C, and the average areas of lesions caused by all species were smaller than in the previous two tests conducted in October and November when the temperatures ranged from 24-27 C. Thus, hotter and dryer conditions and variability within genotypes may have limited the disease reaction, and these conditions may have limited the evaluation of virulence among the fungi.

Variation in lesion size was also observed among replications of an individual treatment (species by cultivar) during a given experiment. This variation in lesion size was probably a result of heterogeneity which is observed commonly in open-pollinated plants.

Usually several isolates of each Phytophthora spp. are used for comparisons of virulence among species to account for the natural variability that is often found among isolates of a species (97, 126, 159). However, intraspecific variations among isolates of P. capsici, P. citrophthora and P. palmivora from Brazil were insignificant (28, 81). Similar results were obtained with comparisons of 12 isolates of P. megakarya as indicated above and three isolates of P. heveae obtained from cacao roots (data not presented). If there is any differential variation in isolates related to the cacao organ attacked, as stated by Medeiros (97), it was not observed in the present experiments. Adequate infection and lesion formation appeared to have been obtained with stems, pods, roots, and leaves inoculated with all of the species in spite of the origin of the isolates. However, only individual isolates of P. capsici, P. citrophthora, and P. palmivora were used in these studies, and the lack of optimum environmental conditions may have prevented differentiation of virulence in tests with isolates of P. heveae and P. megakarya. Additional tests with multiple isolates of all five Phytophthora spp. on various parts of cacao trees will be required to determine accurately the range of pathogenic variability of any character within populations of these fungi.

Variability was noted in responses of tolerance of the three cacao cultivars used in stem inoculations to the

Phytophthora spp. evaluated (Figures 2.3-2.5). For instance, PA 121, SIC 19 and SIC 864 generally showed more tolerance to P. capsici, P. megakarya, and P. heveae, than to P. citrophthora and P. palmivora. These differences in tolerance or resistance to Phytophthora spp. have been encountered often in previous inoculation tests with attached pods from different cultivars of cacao (80, 88). Results of the present study support reports on the behavior of these cultivars in relation to the three Phytophthora spp. that occurred in Bahia, Brazil (80, 88). SIC 864 is a Catongo selection that has been shown to have some resistance to P. capsici but is susceptible to P. palmivora and P. citrophthora (79). Cultivar PA 121 is considered resistant to P. capsici and P. palmivora and moderately resistant to P. citrophthora, whereas SIC 19 is susceptible to all three Phytophthora spp. (88).

Growth of the Phytophthora spp. in excised roots of cacao under controlled conditions correlated well with detached pod inoculations. The excised root technique has the advantage over inoculations with pods or seedling roots of allowing controlled and reproducible assessment of isolates from soil and roots in the type of host tissue in which they normally live. The effects of host resistance or tolerance that may be expressed some time after inoculation can not be assessed well by these types of inoculation tests (138), but factors expressed in the short term, such as

lesion development, can be differentiated (128). The excised root technique may need only to be improved to avoid contamination to be of great use in testing the pathogenicity of Phytophthora spp. isolated from soil and roots. It has the advantage that plant materials for inoculation are always available.

The results of this study show the importance of using all of the Phytophthora spp. that have been shown to cause disease on cacao in a region in screening pods, stems or roots for resistance or tolerance to Phytophthora spp. Consequently, it is of great importance to identify all of the Phytophthora spp. that occur in each cacao growing area of the world. The establishment of strategies for control of diseases caused by Phytophthora spp. in the various areas depends on the identity of the pathogens present, how each develops under local environmental conditions, and their sources of inoculum.

CHAPTER 3
DETERMINATION OF THE RELATIONSHIP OF INOCULUM DENSITY OF PHYTOPHTHORA SPP. IN SOIL TO INCIDENCE OF DISEASE IN CACAO ROOTS

Introduction

Phytophthora spp. have complicated life cycles with strikingly different spore forms that include motile zoospores that are produced in sporangia; thick- or thin-walled, asexual chlamydospores, and thick-walled, sexual oospores (160). Phytophthora capsici, P. citrophthora, P. heveae, P. megakarya, and P. palmivora produce sporangia on naturally infected parts of cacao (Luz, unpublished). These sporangia germinate indirectly and release zoospores, which are considered to be the primary infection units under wet conditions (61). The formation and germination of sporangia, as well as host infection, are regulated by various environmental conditions, but there is no doubt that water and temperature are of primary importance (42).

Chlamydospores, considered to be the most important propagules for the survival of some Phytophthora spp. in soil (163), are produced by four of the five Phytophthora spp. included in this study. Chlamydospores are not produced by isolates of P. capsici from cacao in Brazil (26,

66, 70) nor from pepper in southern Florida (15). The formation and germination of chlamydospores of some Phytophthora spp. are influenced by several variables, among which water potential, the carbon to nitrogen ratio of the substrate, aeration, and temperature appear to be the most important (120).

Oospores of P. capsici, P. palmivora, and P. megakarya are produced when isolates of two opposite compatibility types are paired. These sexual spores are hypothesized to be the primary survival propagules and the primary inoculum source of P. capsici in fields of pepper, squash, and eggplants (15). In cacao, oospores of P. capsici were found in naturally infected pods (69). No other references to natural occurrences of oospores of the other heterothallic species pathogenic to cacao were found. Phytophthora heveae is homothallic and oospores, as well as sporangia, are produced abundantly on naturally infected parts of the cacao tree (87). Isolates of P. citrophthora from cacao do not form oospores (26).

Different optimal environmental conditions are required for the production of the different kinds of inoculum, plant infection, and survival by each species of Phytophthora. Thus, it is important to know the role of each kind of inoculum with respect to infection and disease of cacao roots, and the conditions under which the inoculum can infect the host.

The soil in cacao plantations has been shown to be a reservoir of inoculum of Phytophthora spp. (1, 39, 97, 108, 109, 110, 113, 136, 143, 148, 152). It was suggested that, as is commonly observed with many members of the genus Phytophthora, at least one phase of the life cycle of the species that cause cacao black pod occurs in the soil (111). However, very little is known about the adaptability of these species of Phytophthora to living and surviving in the soil associated with cacao roots.

Root infection by P. palmivora was first reported in Ghana by Turner and Asomaning (145). In Nigeria, Onesirosan (112) isolated a Phytophthora sp. (probably P. megakarya) from dead and living rootlets of cacao as well as from organic debris in the humus layer. An increased importance of Phytophthora spp. as cacao root pathogens has been accepted after the findings of Ward and Griffin (148) in Nigeria. They isolated P. palmivora occasionally and P. megakarya frequently from feeder roots of mature trees during the rainy season. After reviewing the findings of the International Phytophthora Group Project in Nigeria, Gregory et al. (54) hypothesized that root infection can take a very active role in the soil phases of the life cycle of P. megakarya and P. palmivora. Since P. citrophthora attacks roots of other hosts (51, 100, 153), it is not difficult to hypothesize that it also can be a cacao root pathogen. However, very little is known of the comparative

behavior of the different Phytophthora spp. in host roots. Pereira (115) claimed that P. capsici, P. citrophthora, and P. palmivora were isolated from roots of cacao and soil in Brazil, but his work did not allow taxonomic or quantitative differentiation among Phytophthora spp. Roots of cacao seedlings have been infected experimentally with P. palmivora by exposing the roots to suspensions of zoospores (165), sporangia (145), and hyphae (7). Retarded growth of the inoculated young seedlings was observed. Kellam and Zentmyer (70) obtained infection and mortality of cacao seedlings using chlamydospores of P. palmivora and P. citrophthora and oospores of P. capsici as inoculum.

With the exception of work by Kellam and Zentmyer (70), the influence of inoculum density of Phytophthora spp. on incidence and severity of disease in cacao roots has received little attention. Consequently, quantitative information on inoculum density effects on disease is generally lacking for the Phytophthora spp. which are pathogenic to cacao. Mitchell and Kannwischer-Mitchell (103) stressed the need of using forms of inoculum that play important roles in disease development in the field when developing tests on inoculum density-disease incidence relationships; environmental conditions should simulate as closely as possible conditions that occur in the field. For the pathosystems with Phytophthora spp. and cacao roots, these inoculum forms are not known yet, but probably include

chlamydospores, oospores, sporangia, and zoospores. Thus, it is important to elucidate the potential roles that the various propagules of Phytophthora spp. play in initiating infection of cacao roots.

Flooded soil conditions can influence the development of Phytophthora root and crown rots by affecting both the activity of the fungus and the susceptibility of the host (19). Water saturation stimulates the release of zoospores of Phytophthora spp. and can stimulate germination of oospores, chlamydospores, and sporangia in the presence of host roots (74, 89, 132, 133). Anaerobic soil conditions caused by flooding can disrupt the function and structure of roots and increase root exudation that stimulates chemotactic responses of zoospores (41). Flooding has been shown to have a profound effect on disease severity in other plant species infected with several Phytophthora spp. (19, 93, 94, 154).

The objectives of this portion of the research were to determine which forms of inoculum of P. capsici, P. citrophthora, P. heveae, P. megakarya, and P. palmivora play important roles in infection and disease development of cacao seedlings; evaluate the influence of flooding on disease incidence and severity; and determine quantitative responses in infection, disease, and mortality of cacao seedlings to defined levels of inoculum of the five species of Phytophthora.

Materials and Methods

Greenhouse studies were conducted at the University of Florida and at CEPLAC in Brazil to determine the forms of inoculum that can play important roles in root disease. A sandy soil (Myakka sand) at pH 6.6 and 0.95% organic matter was used in experiments conducted with chlamydospores and oospores at the University of Florida. This soil was collected at Delray Beach, Palm Beach County, Florida. Coarse builder's sand was used for tests with zoospores in Florida. In Brazil all experiments were conducted with an alfisol of clay loamy texture (pH 5.9 and 3.2% of organic matter) collected from mature cacao plantations in Bahia. Unless indicated otherwise, soil for all tests was pasteurized for 4 hours at 80 C on each of 2 consecutive days, and maintained for 4 weeks to allow microbial recolonization before use in tests. Soil was infested with the following forms of inoculum: zoospores of each of the five species; chlamydospores of P. citrophthora, P. megakarya, and P. palmivora; fragments of mycelium or oospores produced by crosses between A1 and A2 compatibility types of P. capsici; or homothallicly produced oospores of P. heveae.

Isolates P23 of P. capsici, P62 of P. citrophthora, P208 of P. heveae, Etoun or P613 of P. megakarya, and P26 of P. palmivora were used in all of the inoculation tests

performed in this study. All of the isolates, with the exception of P613, which came originally from Nigeria and was supplied by G.A. Zentmyer from the University of California Culture Collection, were identified in Chapter 2. Oospores of P. capsici were obtained from crosses between P23 (A^1) and P1043 (A^2). Isolate P1043 was obtained from a cacao pod in Bahia, Brazil.

Zoospores were produced as described in Chapter 2. Concentrations of encysted zoospores were determined by counting five fields for 10 samples from each tube of zoospore suspension on a standard hemacytometer. Cacao seedlings (5 to 12 weeks old), obtained from seeds produced in the germplasm fields at CEPLAC, Brazil, were inoculated with zoospores according to the technique described by Mitchell (102, 104). The soil was flooded with tap water, and the zoospore suspension was added to the water surface through a wide-mouth pipet to obtain a final concentration of 10^5, 5 x 10^5 or 10^6 zoospores per plant. Zoospores of P. heveae were included as inoculum for cacao seedlings only in one experiment conducted in Brazil. Each experiment in Brazil and Florida with zoospores of the other four Phytophthora spp. was repeated once.

Chlamydospores were produced in V-8 broth as described by Tsao (140). After 4 weeks at 18 C, each mycelial mat was rinsed on a 44-mm, nylon screen with distilled water and comminuted twice for 30 sec in a Waring blender at high

speed with 25 ml of distilled water (118). Each chlamydospore suspension was homogenized in a tissue grinder and sonicated twice for 30 sec at 240 w. Concentrations of chlamydospores were determined as described above for zoospore suspensions.

Oospores of P. capsici were produced as previously described by Kaosiri et al. (1980). After 5 weeks at 25 C, cultures on V-8 juice broth containing oospores were drained and the mycelial mats washed with 10^{-3} M MES (pH adjusted to 6.2 with 0.1N KOH). Mats were placed in a Waring blender, covered with MES, and blended at high speed for 2-3 min. The suspension was sonicated twice for 30-second periods at 200 w. Cellulase at 1% was added to the suspension at a ratio of 1:1 (v:v) and the suspension was incubated for 24 hours at 25 C in the dark. After this period the oospore suspension was comminuted again, homogenized in a tissue grinder, and sonicated. It was then filtered and centrifuged for 2.5 min at low speed (approximately 1500 rpm). The oospore concentration was determined as described for zoospores. Oospores of P. heveae were produced by the same method described for chlamydospore production, and abundant oospores formed after 3 weeks at 18 C.

Soil was infested with chlamydospores and oospores using the technique described by Ramirez and Mitchell (118) and Kannwischer and Mitchell (64). Portions of the inoculum suspension were added to soil in amounts to give a final

calculated propagule density of 100, 250 or 500 chlamydospores or oospores per gram of soil. The propagules were mixed thoroughly into the soil with a Hobart mixer (The Hobart MFG Co., Troy, Ohio). Five- to eight-week-old cacao seedlings were then planted in infested and noninfested soil.

The initial detectable inoculum density in each lot of infested soil was assayed 24 hours after infestation by dilution plates of subsamples from each lot. Ten grams of infested soil were diluted with 90 ml of 0.25% water agar and 1-ml samples were spread onto each of 10 plates of a selective medium (PARPH, described in Chapter 2) for isolation of Phytophthora spp. from soil (63). Phytophthora megakarya is very sensitive to pimaricin (148) and the amount of this compound was reduced from 10 to 5 mg per liter in the selective medium used to isolate this species. After 2 or 3 days of incubation at room temperature in the dark, soil was washed from the surface of the agar plates and colonies of the Phytophthora spp. were counted (102).

Since dosages of 10^5 zoospores per plant, or 50 and 100 chlamydospores or oospores per gram of soil resulted in low levels of disease and little or no plant mortality in Florida, additional studies were conducted in Brazil to evaluate the effect of 48 hours of flooding each week on disease severity. Seedlings of the cacao cultivars SIC 19 and PA 121 were grown in soil infested with 2×10^5 zoo-

spores of P. capsici, P. citrophthora, P. megakarya or P. palmivora per plant; 200 chlamydospores of P. megakarya or 100 chlamydospores of P. citrophthora or P. palmivora per gram of soil; and with 100 or 250 oospores of P. capsici or P. heveae per gram of soil. One-half of the inoculated plants and non-inoculated controls (10 of each) were subjected to flooding, and the other half were maintained under a daily watering schedule. Immediately after inoculation, and every week thereafter, containers of plants in the flooded treatments were placed in pails of tap water and flooded for 48 hours to a depth of 0.5-1.0 cm above the surface of the soil. They were then removed from the pails and allowed to drain freely for 1 day, after which they were watered for the remainder of the week in the same way as plants that had not been flooded. The plants were kept under greenhouse conditions during and after flooding. Flooded, nonflooded, and noninfested control plants were randomized in a randomized complete block design. Plants were maintained for 8 weeks in a greenhouse with a temperature range of 22 to 26 C.

In most experiments, at both locations, the soil was steam-pasteurized one month before use, but raw soil was evaluated in two experiments in Brazil. Phytophthora palmivora, at 2×10^5 zoospores per plant, was used to inoculate plants in steam-pasteurized and raw soil from the same field.

All of the experiments were performed twice with each form of inoculum and at least 10 plants of each of two cultivars were inoculated with each species at each time. In Florida, each plant was grown in a plastic pot (10.5 cm in diameter) containing 1 kg of soil; in Brazil, each plant was grown in 750 g of soil contained in a plastic bag. In all of the experiments conducted in Brazil, one susceptible cultivar, SIC 19, and one tolerant cultivar, PA 121, were used. In the experiments conducted at the University of Florida, two susceptible cultivars were used. Cultivars SIC 19 and SIAL 325 were used for experiments with zoospores and SIC 23 and ICS 1 were used for experiments with chlamydospores and oospores. Unfortunately, seeds of any one cultivar were not available for all tests.

Seedlings of all of the cultivars were grown in greenhouses at the University of Florida and at CEPLAC in Brazil. The seeds, all obtained from natural open pollination in the germplasm fields in Brazil, were sown in plastic trays containing builders sand that had been autoclaved for 2 hours on each of two successive days. A week after the seedlings emerged they were transplanted to plastic pots containing autoclaved sand. At the University of Florida, the sand in which the seedlings were grown was infested with zoospores by the procedure described previously.

The numbers of wilted plants in all treatments and experiments were recorded. The root systems of wilted, moribund plants were plated on the selective medium to confirm the presence of Phytophthora spp. Eight weeks after planting, the remaining plants of all of the experiments were harvested. The root systems of the plants were washed and weighed, and the incidence of root infection was assessed as the percentage of 20 plants from which Phytophthora spp. was recovered on PARPH.

As the root systems of cacao plants 3 or 4 months old were well developed, the roots were divided into several parts following an approximation of the morphometric system of root analysis developed by Fitter (50). According to Fitter's scheme, root branching is defined from apical meristems inward. Any root which terminates in an apical meristem is defined as a first-order root. Where two first-order roots merge, there begins a second-order root. Where two second-order roots merge, there begins a third-order root and so forth. The union of a lower order root element with that of a higher order does not alter the classification of the highest order root. Within this dynamic scheme, increasing levels of root order are related to increasing tissue maturity.

Roots in the different categories were immersed in 70 % ethanol for 30 seconds, rinsed in sterile water, and plated separately on a selective medium to determine in which root

class infection was most prevalent. The plates were observed for growth of the pathogen after 2 or 3 days of incubation in the dark; colonies of Phytophthora spp. growing from the roots of each category were counted. Since colonies of P. megakarya grew more slowly on PARPH than those of the other Phytophthora spp., colonies of the former fungus had to be counted 3 or 4 days after plating the root systems.

A disease severity index based on a scale of 0 to 5 was developed according to the number of colonies growing per root category. The scale of the disease severity index was: 0, no infection; 1, one to four first-order roots infected; 2, five to seven first-order roots infected; 3, one to two second-order roots infected or more than seven first-order roots infected; 4, more than two second-order roots infected, one or more colonies on tap root, or one or more cankers on tap root; and 5, plant moribund (wilted) or dead.

The data for each cultivar in each experiment were analyzed individually as a two-factor, factorial experiment in a completely randomized block design. The two-factor interactions analyzed were species and inoculum dosages. For the flooding experiments, the two-factor interactions were flooding (flooded and nonflooded) and species. The flooding experiment with soil infested with oospores of P. capsici and P. heveae was analyzed as a three-factor factorial; flooding, species, and inoculum dosage were the three factors. The three variables analyzed in each set of

data were root weight, number of colonies per root system, and disease severity index. Because too many zeros occurred in the data array, percentages of infection and mortality were not statistically analyzed. Duncan's multiple range test was used to compare means of the treatments comprising infection with different species but only over a single inoculum level; a t test (Least Significant Difference) was used to compare the mean of each treatment with the control mean in experiments involving more than one inoculum dosage. Dunnett's test was used to compare means of treatments with more than one inoculum level within an individual species.

Results

The germination of zoospores from inoculum suspensions after 24 hours of incubation on water agar ranged from 90 to 95% for P. palmivora, 84 to 90% for P. citrophthora, 92 to 97% for P. capsici, 80 to 85% for P. megakarya, and 89 to 92% for P. heveae (Table A-1, Appendix). Initial recovery of P. palmivora and P. citrophthora from infested soil with chlamydospores was usually within 95% of the calculated infestation densities of 100, 250 or 500 chlamydospores per gram of soil, but P. megakarya was recovered at 72-80% of the levels used for infestation (Table A-2, Appendix). Oospores of P. capsici did not germinate on PARPH after 24 hours of incubation in soil, whereas 27-34% germination was

obtained with oospores of P. heveae (Table A-3, Appendix).

Although raw soil was not used routinely in this study, the root weights of plants of two cultivars growing in infested and noninfested raw soil were similar in two tests to values obtained for plants growing under the same conditions in steam-pasteurized soil (Table 3.1). However, the average numbers of colonies of P. palmivora growing from the root systems of plants of PA 121 and SIC 19 were always greater in pasteurized than in raw soil, and colonies of Phytophthora spp. were also observed growing from the root system of control plants in raw soil. The presence of indigenous populations of pythiaceous fungi necessitated the use of pasteurized soil in the subsequent experiments.

Few significant differences in any of the disease measurements occurred between nonflooded and flooded treatments when zoospores or chlamydospores were used for inoculum (Tables 3.2-3.5). A significant effect of the interaction of species and flooding treatment was observed for experiments with zoospores only with cultivar PA 121 for variables of root weight and number of colonies per root system; in chlamydospore experiments, the interaction was significant with both cultivars for severity index and also with PA 121 for number of colonies per root system. Fewer colonies of P. palmivora were observed on roots of PA 121 plants after inoculation with zoospores and flooding than on non-flooded roots (Table 3.2). Percentages of infection and

Table 3-1. Effect of soil pasteurization on root weight and disease incidence 8 weeks after planting 8-week-old seedlings of the cacao cultivars SIC 19 and PA 121 in soil infested with $2x10^5$ zoospores of Phytophthora palmivora per plant.

Cultivars		Root weight[w] (g)		# Colonies/ root system[w,x]		# Colonies from indigenous fungi[y]	
		P[z]	NP[z]	P	NP	P	NP
PA 121	I[z]	4.47bc	4.08c	10.3a	6.6b	0	4.8
	C[z]	5.22ab	5.48a	0.0c	0.5c	0	4.9
SIC 19	I	3.31a	3.51a	14.1a	6.7b	0	5.7
	C	2.62b	2.75b	0.0c	0.5c	0	4.6

[w]Each value is an average of 20 replications. Means followed by the same letter are not significantly different according to Duncan's multiple range test ($P<0.01$). Comparisons among means for each cultivar are valid for each disease parameter.
[x]Average of total number of colonies of P. palmivora per root system as determined by plating roots on a selective medium.
[y]Average of total number of colonies of other pythiaceous fungi per root system.
[z]P=pasteurized soil; NP=nonpasteurized soil; I=infested soil; C=noninfested soil.

Table 3-2. Comparisons of the effect of flooding on disease incidence, disease severity, and percentages of infection and mortality 8 weeks after planting 7-week-old seedlings of the cacao cultivar PA 121 in soil infested with $2x10^5$ zoospores of Phytophthora citrophthora, P. palmivora, P. capsici or P. megakarya per plant.

Species	Root weight[u] (g)		# Colonies/ root system[u,w]		Severity index[u,x]		% Infection[y]		% Mortality[z]	
	F[v]	NF[v]	F	NF	F	NF	F	NF	F	NF
P. citrophthora	1.88bc	1.94bc	9.2a	8.9a	3.3a	3.0a	100	100	30	20
P. palmivora	1.97bc	1.67c	6.4b	9.2a	2.5a	3.2a	100	100	10	30
P. capsici	2.32ab	2.01bc	1.8c	2.2c	0.6b	0.7b	60	70	0	0
P. megakarya	2.71a	2.66a	0.4c	0.6c	0.3b	0.4b	30	40	0	0
Control	2.18b	2.18b	0.0c	0.0c	0.0b	0.0b	0	0	0	0

[u]Average root weight of 20 plants per treatment; means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.05$). Comparisons were valid for means in columns and rows for each disease parameter.

[v]F=Plants were flooded for 48 hours each week; NF=Plants were not flooded.

[w]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.

[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.

[y]Infection, expressed as the percentage of 20 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.

[z]Mortality is expressed as the percentage of 20 inoculated plants that had died 8 weeks after inoculation.

Table 3-3. Comparisons of the effect of flooding on disease incidence, disease severity, and percentages of infection and mortality, eight weeks after planting 7-week-old seedlings of the cacao cultivar SIC 19 in soil infested with $2x10^5$ zoospores of Phytophthora citrophthora, P. palmivora, P. capsici or P. megakarya per plant.

Species	Root weight[u] (g)		# Colonies/ root system[u,w]		Severity index[u,x]		% Infection[y]		% Mortality[z]	
	F[v]	NF[v]	F	NF	F	NF	F	NF	F	NF
P. citrophthora	1.28bc	1.17c	12.1a	12.1a	4.3a	4.3a	100	100	50	50
P. palmivora	1.09c	1.18c	12.1a	12.6a	4.2a	4.1a	100	100	40	40
P. capsici	1.34abc	1.82a	5.6b	4.8b	2.0b	1.6b	95	100	0	0
P. megakarya	1.77a	1.69ab	1.3c	1.3c	0.6c	0.5c	60	50	0	0
Control	1.82a	1.55abc	0.0c	0.0c	0.0c	0.0c	0	0	0	0

[u]Average of root weight of 20 plants per treatment; means followed by the same letter were not significantly different according to Duncan's multiple range test (P0.01). Comparisons were valid for means in columns and rows for each disease parameter.
[v]F=Plants were flooded 48 hours weekly; NF=Plants were not flooded.
[w]Average of the total number of colonies obtained per root system as determined by plating on a selective medium.
[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.
[y]Infection, expressed as the percentage of 20 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.
[z]Mortality is expressed as the percentage of the 20 inoculated plants that had died 8 weeks after inoculation.

Table 3-4. Comparisons of the effect of flooding on disease incidence, disease severity, and percentages of infection and mortality 8 weeks after planting 7-week-old seedlings of the cacao cultivar PA 121 in soil infested with 100 chlamydospores of Phytophthora citrophthora or P. palmivora or 200 chlamydospores of P. megakarya per gram of soil.

Species	Root weight[u] (g)		# Colonies/ root system[u,w]		Severity index[u,x]		% Infection[y]		% Mortality[z]	
	F[v]	NF[v]	F	NF	F	NF	F	NF	F	NF
P. citrophthora	2.04c	2.07c	10.7a	9.2ab	3.1a	2.5ab	100	100	0	0
P. palmivora	2.09c	2.15c	6.7bc	7.8b	1.9bc	2.3b	100	100	0	0
P. megakarya	2.70b	2.79b	4.2cd	3.5d	1.4cd	1.1d	100	75	0	0
Control	3.27a	3.53a	0.0e	0.0e	0.0e	0.0e	0	0	0	0

[u]Average root weight of 20 plants per treatment; means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.01$). Comparisons were valid for means in columns and rows for each disease parameter.
[v]F=Plants were flooded 48 hours weekly; NF=Plants were not flooded.
[w]Average of the total number of colonies obtained per root system as determined by plating roots on a selective medium.
[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.
[y]Infection, expressed as the percentage of 20 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.
[z]Mortality is expressed as the percentage of 20 inoculated plants that had died 8 weeks after inoculation.

Table 3-5. Comparisons of the effect of flooding on disease incidence, disease severity, and percentages of infection and mortality 8 weeks after planting 7-week-old seedlings of the cacao cultivar SIC 19 in soil infested with 100 chlamydospores of Phytophthora citrophthora or P. palmivora or 200 chlamydospores of P. megakarya per gram of soil.

Species	Root weight[u] (g)		# Colonies/ root system[u,w]		Severity index[u,x]		% Infection[y]		% Mortality[z]	
	F[v]	NF[v]	F	NF	F	NF	F	NF	F	NF
P. citrophthora	1.03b	1.15b	19.6a	17.5a	4.7a	4.6ab	100	100	70	60
P. palmivora	1.30b	1.29b	11.1b	10.9b	4.2ab	4.2ab	100	100	35	25
P. megakarya	1.86a	1.92a	6.4c	5.5c	2.1c	2.4c	100	100	0	0
Control	2.05a	2.17a	0.0d	0.0d	0.0d	0.0d	0	0	0	0

[u]Average of root weight of 20 plants per treatment; means followed by the same letter were not significantly different according to Duncan's multiple range test (P<0.01). Comparisons were valid for means in columns and rows for each disease parameter.
[v]F=Plants were flooded 48 hours weekly; NF=Plants were not flooded.
[w]Average of the total number of colonies obtained per root system as determined by plating roots on a selective medium.
[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.
[y]Infection, is expressed as the percentage of 20 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.
[z]Mortality is expressed as the percentage of the 20 inoculated plants that had died 8 weeks after inoculation.

mortality were also similar in the flooded and nonflooded treatments. Severity of disease and numbers of colonies per root system were generally greater in both cultivars exposed to zoospores or chlamydospores of P. citrophthora and P. palmivora than in those exposed to P. capsici or P. megakarya. Plant mortality in these tests occurred only with plants inoculated with the former two fungi. Infection of roots by zoospores of P. megakarya, when compared to the controls, resulted in no disease expression, but roots infected in chlamydospore-infested soil had significantly greater numbers of colonies per root system and disease severity than that observed in the non-infected control plants (Tables 3.4 and 3.5). However, 200 chlamydospores of P. megakarya per gram of soil were used, which was twice the inoculum level used to infest soil with P. citrophthora or P. palmivora.

The percentages of plants infected by P. capsici and P. heveae were greater in flooded than in non-flooded treatments at 100 oospores per gram of soil with both cultivars and at 250 oospores per gram of soil with PA 121 (Table 3.6). For these experiments the interaction of species and flooding treatment was generaly highly significant for both cultivars. Both pathogens killed some of the flooded plants of SIC 19 at both inoculum levels, as well as nonflooded plants at 250 oospores per gram of soil (Table 3.7). None of the PA 121 plants died. Reductions in root weights of

Table 3-6. Comparisons of the effect of flooding on disease incidence, disease severity, and percentages of infection and mortality 8 weeks after planting 7-week-old seedlings of the cacao cultivar PA 121 in soil infested with 100 or 250 oospores of Phytophthora capsici or P. heveae per gram of soil.

Species		Root weight[u] (g)		# Colonies/ root system[u,w]		Severity index[u,x]		% Infection[y]		% Mortality[z]	
		F[v]	NF[v]	F	NF	F	NF	F	NF	F	NF
P. capsici	100	2.62ab	2.71ab	0.7*b	0.2*b	0.5*b	0.2*b	50	20	0	0
P. heveae	100	2.52b	2.85*a	7.6a	1.8*b	2.6a	0.6*b	100	60	0	0
P. capsici	250	2.51a	2.65a	2.7bc	1.3*c	1.1*b	0.6*b	95	75	0	0
P. heveae	250	2.52a	2.78a	9.3a	3.6b	2.9a	1.2*b	100	70	0	0
Control		3.00	3.14	0.0	0.0	0.0	0.0	0	0	0	0

[u]Average root weight of 20 plants per treatment; means followed by the same letter were not significantly different according to Duncan's multiple range test (P<0.01). Comparisons were valid for means in columns and rows for each disease parameter and the same inoculum level. Means followed by an asterisk were not significantly different from the control according to Dunnett's test (P<0.01).

[v]F=Plants were flooded 48 hours weekly; NF=Plants were not flooded.

[w]Average of the total number of colonies obtained per root system as determined by plating roots on a selective medium.

[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.

[y]Infection, expressed as the percentage of 20 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.

[z]Mortality is expressed as the percentage of 20 inoculated plants that had died 8 weeks after inoculation.

plants in flooded, as compared to non-flooded, treatments occurred with 100 oospores per gram of soil for P. capsici on SIC 19, for P. heveae on both cultivars, and with 250 oospores per gram of soil with both pathogens on SIC 19. Disease was more severe and more colonies per root system were observed in flooded, as compared to non-flooded, plants with P. heveae at both inoculum levels on both cultivars, and with P. capsici at 100 oospores per gram of soil on SIC 19. The severity index was also greater for flooded compared to non-flooded plants of SIC 19 with P. capsici at 250 oospores per gram of soil. Phytophthora heveae formed more colonies on roots and caused more severe damage to roots of seedlings of both cultivars under flooded conditions at both inoculum levels tested than did P. capsici, except with 250 oospores per gram of soil on SIC 19 for the severity index. Few or no significant differences were observed between the two species in the non-flooded treatments.

Mortality of plants in the three cultivars inoculated with zoospores in Florida and in Brazil was limited to plants infected with P. citrophthora, P. palmivora, and occasionally P. capsici (Tables 3.8-3.11). Plant mortality was greater at the higher inoculum levels, and P.

Table 3-7. Comparisons of the effect of flooding on disease incidence, disease severity, and percentages of infection and mortality 8 weeks after planting 7-week-old seedlings of the cacao cultivar SIC 19 in soil infested with 100 or 250 oospores of Phytophthora capsici or P. heveae per gram of soil.

Species		Root weight[u] (g)		# Colonies/ root system[u,w]		Severity index[u,x]		% Infection[y]		% Mortality[z]	
		F[v]	NF[v]	F	NF	F	NF	F	NF	F	NF
P. capsici	100	1.49*b	1.79*a	4.8b	0.9*c	1.9b	0.5*c	100	50	10	0
P. heveae	100	1.40b	1.80*a	7.6a	1.0*c	3.0a	0.4*c	100	40	20	0
P. capsici	250	0.73b	1.12a	8.5b	8.2b	4.5ab	3.6c	100	100	70	40
P. heveae	250	0.78b	1.17a	11.5a	9.5b	4.8a	4.0bc	100	100	80	50
Control		1.76	1.91	0.0	0.0	0.0	0.0	0	0	0	0

[u]Average root weight of 20 plants per treatment; means followed by the same letter were not significantly different according to Duncan's multiple range test (P<0.01). Comparisons were valid for means in columns and rows for each disease parameter and the same inoculum level. Means followed by an asterisk were not significantly different from the control according to Dunnett's test (P<0.01).

[v]F=Plants were flooded 48 hours weekly; NF=Plants were not flooded.

[w]Average of the total number of colonies obtained per root system as determined by plating roots on a selective medium.

[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.

[y]Infection, expressed as the percentage of 20 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.

[z]Mortality is expressed as the percentage of 20 inoculated plants that had died 8 weeks after inoculation.

Table 3-8. Root weight, disease incidence, disease severity, and percentages of infected plants and mortality 8 weeks after planting 8-week-old cacao seedlings of the cultivar PA 121 in soil infested with different densities of zoospores of Phytophthora citrophthora, P. palmivora, P. capsici, or P. megakarya per plant.

Species	ID	Root[v] weight (g)	# Colonies/ root syst.[w]	Severity index[x]	% Infect[y]	% Mort[z]
Experiment 1[u]						
P. citrophthora	$5x10^5$	2.04 b	12.2 a	3.9 a	100	20
P. palmivora	$5x10^5$	4.07 a	9.3 a	2.7 b	100	0
P. capsici	$5x10^5$	3.70 ab	5.2 b	1.8 c	80	0
P. megakarya	$5x10^5$	4.54*a	0.1*c	0.1*d	10	0
P. citrophthora	10^6	2.11 c	17.6 a	4.4 a	100	40
P. palmivora	10^6	2.89 bc	14.7 a	3.8 a	100	20
P. capsici	10^6	2.48 c	9.3 b	3.5 a	100	30
P. megakarya	10^6	4.18 a	0.7*c	0.4*b	40	0
Control		5.24	0.0	0.0	0	0
Experiment 2						
P. citrophthora	$5x10^5$	4.18 b	9.4 a	3.2 a	100	10
P. palmivora	$5x10^5$	4.14 b	10.0 a	2.8 a	100	0
P. capsici	$5x10^5$	5.77*a	5.9 b	1.9 b	100	0
P. megakarya	$5x10^5$	4.63 ab	0.5*c	0.3*c	30	0
P. heveae	$5x10^5$	4.84 ab	2.3*c	0.7*c	60	0
P. citrophthora	10^6	3.82 ab	10.8 a	4.0 a	100	30
P. palmivora	10^6	3.42 b	11.8 a	3.4 ab	100	10

Table 3-8. Continued.

Species	ID	Root[v] weight (g)	# Colonies/ root syst.[w]	Severity index[x]	% Infect[y]	% Mort[z]
P. capsici	10^6	4.90 a	9.6 a	3.0 b	100	0
P. megakarya	10^6	3.72 ab	1.9*c	0.6*d	60	0
P. heveae	10^6	4.34 ab	6.6 b	2.2 c	100	0
Control		6.75	0.0	0.0	0	0

[u]Both experiments were conducted at CEPLAC, Brazil.
[v]Each value in this column is the average of root weights of 10 plants. Means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.01$). Comparisons were valid within the same column only for means at each inoculum dosage. Means in each column followed by an asterisk were not different from the control according to Dunnett's test ($P<0.01$).
[w]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.
[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.
[y]Infection, expressed as the percentage of 20 inoculated plants that were infected 8 weeks after inoculation, was determined by by plating roots on a selective medium.
[z]Mortality is expressed as the percentage of 20 inoculated plants that had died 8 weeks after inoculation.

Table 3-9. Root weight, disease incidence, disease severity, and percentages of infected plants and mortality 8 weeks after planting 8-week-old cacao seedlings of the cultivar SIC 19 in soil infested with different densities of zoospores of Phytophthora citrophthora, P. palmivora, P. capsici, or P. megakarya per plant.

Species	ID	Root[v] weight (g)	# Colonies/ root syst.[w]	Severity index[x]	% Infect[y]	% Mort[z]
Experiment 1[u]						
P. citrophthora	$5x10^5$	2.34 b	10.9 b	3.9 a	100	30
P. palmivora	$5x10^5$	3.37*a	17.0 a	3.5 a	100	0
P. capsici	$5x10^5$	3.49*a	11.3 b	3.3 a	100	0
P. megakarya	$5x10^5$	3.83*a	0.4*c	0.3*b	30	0
P. citrophthora	10^6	1.85 b	14.0 a	4.6 a	100	80
P. palmivora	10^6	2.26 b	17.4 a	4.5 a	100	50
P. capsici	10^6	2.25 b	17.1 a	4.3 a	100	40
P. megakarya	10^6	3.59*a	3.4*b	1.2 b	80	0
Control		3.96	0.0	0.0	0	0
Experiment 2						
P. citrophthora	$5x10^5$	2.26 b	10.1 a	4.0 a	100	20
P. palmivora	$5x10^5$	3.78*a	14.5 a	3.5 ab	100	0
P. capsici	$5x10^5$	3.26 a	11.6 a	3.1 b	100	0
P. megakarya	$5x10^5$	3.46 a	1.5*c	0.7 d	70	0
P. heveae	$5x10^5$	3.97*a	5.4 b	1.8 c	100	0
P. citrophthora	10^6	2.28 c	10.9 b	4.5 a	100	60
P. palmivora	10^6	2.58 bc	15.8 a	4.3 a	100	30

Table 3-9 Continued.

Species	ID	Root[v] weight (g)	# Colonies/ root syst.[w]	Severity index[x]	% Infect[y]	% Mort[z]
P. capsici	10^6	2.54 bc	13.2 ab	4.0 a	100	20
P. megakarya	10^6	3.48 ab	3.3*c	1.2 c	100	0
P. heveae	10^6	3.98*a	9.6 b	2.9 b	100	0
Control		4.78	0.0	0.0	0	0

[u]Both experiments were conducted at CEPLAC, Brazil.
[v]Each value in this column is the average of root weights of 10 plants. Means followed by the same letter were not significantly different according to Duncan's multiple range test (P<0.01). Comparisons were valid within the same column only for means at each inoculum dosage. Means in each column followed by an asterisk were not different from the control according to Dunnett's test (P<0.01).
[w]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.
[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.
[y]Infection, expressed as the percentage of 20 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.
[z]Mortality is expressed as the percentage of 20 inoculated plants that had died 8 weeks after inoculation.

Table 3-10. Root weight, disease incidence, disease severity and percentages of infected plants and mortality 8 weeks after planting 8-week-old cacao seedlings of the cultivar SIC 19 in soil infested with different densities of zoospores of Phytophthora citrophthora, P. palmivora, P. capsici, or P. megakarya per plant.

Species	ID	Root[v] weight (g)	# Colonies/ root syst.[w]	Severity index[x]	% Infect[y]	% Mort[z]
Experiment 1[u]						
P. citrophthora	10^5	1.53*ab	1.4*a	0.6*a	40	0
P. palmivora	10^5	1.37*b	1.8*a	0.7*a	50	0
P. capsici	10^5	1.45*ab	0.6*a	0.2*a	20	0
P. megakarya	10^5	1.79*a	0.0*a	0.0*a	0	0
P. citrophthora	$5x10^5$	1.28 a	8.1 a	2.3 ab	90	0
P. palmivora	$5x10^5$	1.30 a	5.4 ab	2.9 a	90	20
P. capsici	$5x10^5$	1.45*a	3.2*bc	1.3*bc	40	0
P. megakarya	$5x10^5$	1.44*a	0.0*c	0.0*c	0	0
P. citrophthora	10^6	0.89 c	12.5 a	4.8 a	100	80
P. palmivora	10^6	1.14 bc	13.5 a	4.0 a	100	50
P. capsici	10^6	1.31 ab	5.0 b	2.3 b	70	0
P. megakarya	10^6	1.57*a	4.1*b	1.5*b	90	0
Control		1.75	0.0	0.0	0	0
Experiment 2						
P. citrophthora	10^5	1.21*a	1.6*a	0.5*a	40	0
P. palmivora	10^5	1.25*a	1.9*a	0.8*a	60	0
P. capsici	10^5	1.23*a	0.4*a	0.2*a	20	0

Table 3-10 Continued.

Species	ID	Root[v] weight (g)	# Colonies/ root syst.[w]	Severity index[x]	% Infect[y]	% Mort[z]
P. megakarya	10^5	1.26*a	0.0*a	0.0*a	0	0
P. citrophthora	$5x10^5$	1.10 ab	8.6 a	2.7 a	90	0
P. palmivora	$5x10^5$	0.98 b	5.9 b	2.9 a	90	40
P. capsici	$5x10^5$	1.19*ab	3.2*c	1.1*b	50	10
P. megakarya	$5x10^5$	1.26*a	0.8*c	0.4*b	20	0
P. citrophthora	10^6	0.72 b	16.2 a	4.8 a	100	80
P. palmivora	10^6	1.11 a	7.6 b	3.3 b	100	50
P. capsici	10^6	1.10 a	3.4 c	1.3*c	60	10
P. megakarya	10^6	1.26*a	4.4 c	1.8 c	80	0
Control		1.50	0.0	0.0	0	0

[u]Both experiments were conducted at the University of Florida.
[v]Each value in this column is the average of root weights of 10 plants. Means followed by the same letter were not significant ly different according to Duncan's multiple range test ($P<0.01$). Comparisons were valid within the same column only for means at each inoculum dosage. Means in each column followed by an ast erisk were not different from the control according to Dunnett's test ($P<0.01$).
[w]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.
[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.
[y]Infection, expressed as the percentage of 20 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.
[z]Mortality is expressed as the percentage of 20 inoculated plants that had died 8 weeks after inoculation.

Table 3-11. Root weight, disease incidence, disease severity and percentages of infected plants and mortality 8 weeks after planting 8-week-old cacao seedlings of the cultivar SIAL 325 in soil infested with different densities of zoospores of Phytophthora citrophthora, P. palmivora, P. capsici, or P. megakarya per plant.

Species	ID	Root[v] weight (g)	# Colonies/ root syst.[w]	Severity index[x]	% Infect[y]	% Mort[z]
Experiment 1[u]						
P. citrophthora	10^5	1.37*b	1.7*a	0.6*a	40	0
P. palmivora	10^5	1.75*ab	0.7*a	0.3*a	30	0
P. capsici	10^5	1.39*ab	1.2*a	0.5*a	50	0
P. megakarya	10^5	1.82*a	0.0*a	0.0*a	0	0
P. citrophthora	$5x10^5$	1.28*b	6.1 a	2.1 a	80	0
P. palmivora	$5x10^5$	1.60*ab	2.1*b	0.9*b	70	0
P. capsici	$5x10^5$	1.55*ab	2.3*b	0.9*b	50	0
P. megakarya	$5x10^5$	1.85*a	0.6*b	0.4*b	40	0
P. citrophthora	10^6	1.18*a	12.8 a	4.4 a	100	50
P. palmivora	10^6	1.39*a	8.0 b	3.0 b	100	20
P. capsici	10^6	1.26*a	5.7 bc	3.0 b	100	40
P. megakarya	10^6	1.49*a	3.7*c	1.5 c	80	0
Control		1.44	0.0	0.0	0	0
Experiment 2						
P. citrophthora	10^5	1.42*a	2.0*a	0.7*a	40	0
P. palmivora	10^5	1.40*a	0.6*a	0.3*a	30	0
P. capsici	10^5	1.27*a	1.5*a	0.6*a	50	0

Table 3-11 Continued.

Species	ID	Root[v] weight (g)	# Colonies/ root syst.[w]	Severity index[x]	% Infect[y]	% Mort[z]
P. megakarya	10^5	1.38*a	0.0*a	0.0*a	0	0
P. citrophthora	$5x10^5$	1.23*a	7.5 a	2.3 a	80	0
P. palmivora	$5x10^5$	1.16*a	1.8*b	0.9*b	70	0
P. capsici	$5x10^5$	1.18*a	2.0*b	0.8*b	70	0
P. megakarya	$5x10^5$	1.34*a	0.9*b	0.7*b	30	0
P. citrophthora	10^6	1.04 a	13.7 a	4.5 a	100	50
P. palmivora	10^6	1.21*a	7.5 b	3.1 b	100	20
P. capsici	10^6	1.19*a	2.7*c	1.4 c	80	0
P. megakarya	10^6	1.29*a	4.3 c	1.9 c	80	0
Control		1.45	0.0	0.0	0	0

[u]Both experiments were conducted at the University of Florida.
[v]Each value in this column is the average of root weights of 10 plants. Means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.01$). Comparisons were valid within the same column only for means at each inoculum dosage. Means in each column followed by an asterisk were not different from the control according to Dunnett's test ($P<0.01$).
[w]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.
[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.
[y]Infection, expressed as the percentage of 20 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.
[z]Mortality is expressed as the percentage of 20 inoculated plants that had died 8 weeks after inoculation.

citrophthora generally caused the highest incidences of death. Incidence of infection with all Phytophthora spp. increased with the three increasing levels of zoospores in the tests in Florida (Tables 3.10 and 3.11). Phytophthora megakarya was the only species that did not infect plants at 10^5 zoospores per plant, and at 5×10^5 zoospores per plant only low levels of infection were observed. Most of the plants inoculated with P. capsici, P. citrophthora and P. palmivora in Brazil, had high levels of infection even at the lowest concentration of zoospores (Tables 3.8 and 3.9). Generally the root weights of plants were much lower in tests conducted in Florida than in those done in Brazil. These differences were especially evident with SIC 19 which was used in experiments conducted at both locations (Tables 3.9 and 3.10). Except for one test in which plants were infected with P. citrophthora at 10^6 zoospores per plant, the root weights of infected plants of SIAL 325 were not different from those of the control plants (Table 3.11). Phytophthora megakarya generally did not cause reduction of root weights of any of the cultivars when compared to the controls, except with PA 121 . Phytophthora citrophthora reduced root weights of plants, as compared to controls, in all tests at 5×10^5 or 10^6 zoospores per plant for cultivars PA 121 or SIC 19. Lower root weights of these two cultivars also occurred with 10^6 zoospores of P. capsici or P. palmivora per plant. Fewer colonies per root system were

generally formed by P. megakarya than by P. citrophthora, P. capsici, P. palmivora, or P. heveae in tests conducted in Brazil, but similar numbers of colonies occurred with P. megakarya and P. capsici in Florida. Based on the disease severity index with all three cultivars, disease severity was generally lowest in all tests with P. megakarya. Phytophthora heveae caused slightly but significantly more disease than P. megakarya, but the other three species of Phytophthora caused the highest levels of disease.

Seedlings of the cacao cultivars PA 121, SIC 19, SIC 23, and ICS 1 were infected when grown in soil infested with chlamydospores of P. citrophthora, P. palmivora, or P. megakarya (Tables 3.12-3.15). However, infection and disease varied with the species, inoculum level, and cultivar tested. Variations between tests with the same cultivar were also observed. Percentages of infection by P. megakarya increased with increased densities of chlamydospores, but high percentages (70-100%) of plants were infected by P. citrophthora and P. palmivora even at the lowest inoculum density used. No plants were killed by P. megakarya, and percentages of mortality were generally higher with P. citrophthora than with P. palmivora. Mortality was greater with the latter two fungi at 250 as compared to 100 chlamydospores per gram of soil, but employing the higher level of 500 chlamydospores per gram of

Table 3-12. Root weight, disease incidence, disease severity and percentages of infected plants and mortality 8 weeks after planting 5-week-old cacao seedlings of the cultivar PA 121 in soil infested with different densities of chlamydospores of Phytophthora citrophthora, P. palmivora or P. megakarya per gram of soil.

Species	ID	Root[v] weight (g)	# Colonies root syst[w]	Severity index[x]	% Infect[y]	% Mort[z]
Experiment 1[u]						
P. citrophthora	100	2.35*a	2.6*a	0.9*a	70	0
P. palmivora	100	2.59*a	2.8*a	1.1 a	80	0
P. megakarya	100	2.84*a	0.5*a	0.2*a	20	0
P. citrophthora	250	1.29 b	9.3 a	3.4 a	100	30
P. palmivora	250	1.56 b	7.7 a	3.6 a	100	30
P. megakarya	250	2.58*a	2.0*b	0.8*b	50	0
Control		2.65	0.0	0.0	0	0
Experiment 2						
P. citrophthora	100	2.23 b	8.0 a	2.6 a	100	0
P. palmivora	100	2.53*a	4.9 b	1.5 b	100	0
P. megakarya	100	2.66*a	0.4*c	0.3*c	30	0
P. citrophthora	250	1.72 b	11.7 a	3.7 a	100	20
P. palmivora	250	2.20 a	10.9 a	3.2 a	100	10
P. megakarya	250	2.42*a	3.0 b	1.0*b	70	0
Control		2.77	0.0	0.0	0	0

[u]Both experiments were conducted at CEPLAC, Brazil.
[v]Each value in this column is the average of root weights of 10 plants. Means followed by the same letter were not significantly different according to Duncan's multiple range

Table 3-12 Continued.

test ($P<0.01$). Comparisons were valid within the same column only for means at each inoculum dosage. Means in each column followed by an asterisk were not different from the control according to Dunnett's test ($P<0.01$).

[W]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.

[X]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.

[Y]Infection, expressed as the percentage of 20 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.

[Z]Mortality was expressed as the percentage of 20 inoculated plants that had died 8 weeks after inoculation.

Table 3-13. Root weight, disease incidence, disease severity and percentages of infected plants and mortality 8 weeks after planting 5-week-old cacao seedlings of the cultivar SIC 19 in soil infested with different densities of chlamydospores of Phytophthora citrophthora, P. palmivora or P. megakarya per gram of soil.

Species	ID	Root[v] weight (g)	# Colonies root syst[w]	Severity index[x]	% Infec[y]	% Mort[z]
Experiment 1[u]						
P. citrophthora	100	1.12*a	6.9 a	3.7 a	100	40
P. palmivora	100	1.22*a	8.5 a	3.8 a	100	20
P. megakarya	100	1.55*a	1.3*b	0.4*b	40	0
P. citrophthora	250	0.76 b	6.9 b	4.7 a	100	70
P. palmivora	250	0.99*a	11.3 a	4.5 a	100	50
P. megakarya	250	1.36*a	4.2 b	1.5 b	80	0
Control		1.47	0.0	0.0	0	0
Experiment 2						
P. citrophthora	100	1.48*b	10.4 a	3.4 a	100	20
P. palmivora	100	1.58*a	9.1 a	2.7 a	100	0
P. megakarya	100	1.79*a	1.1*b	0.5*b	50	0
P. citrophthora	250	1.15 b	13.5 a	4.4 a	100	50
P. palmivora	250	1.31*b	10.9 b	3.7 a	100	20
P. megakarya	250	1.75*a	2.9 c	1.0 b	80	0
Control		2.41	0.0	0.0	0	0

Table 3-13 Continued.

[u]Both experiments were conducted at CEPLAC, Brazil.
[v]Each value in this column is the average of root weights of 10 plants. Means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.01$). Comparisons were valid within the same column only for means at each inoculum dosage. Means in each column followed by an asterisk were not different from the control according to Dunnett's test ($P<0.01$).
[w]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.
[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.
[y]Infection, expressed as the percentage of 20 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.
[z]Mortality is expressed as the percentage of 20 inoculated plants that had died 8 weeks after inoculation.

Table 3-14. Root weight, disease incidence, disease severity and percentages of infected plants and mortality 8 weeks after planting 5-week-old cacao seedlings of the cultivar SIC 23 in soil infested with different densities of chlamydospores of Phytophthora citrophthora, P. palmivora or P. megakarya per gram of soil.

Species	ID	Root weight[v] (g)	# Colonies root syst[w]	Severity index[x]	% Infect[y]	% Mort[z]
Experiment 1[u]						
P. citrophthora	100	2.86*a	19.8 a	3.9 a	100	20
P. palmivora	100	3.43*a	9.3 b	2.7 b	90	0
P. megakarya	100	3.52*a	0.5*c	0.2*c	20	0
P. citrophthora	250	2.51 a	26.7 a	4.5 a	100	50
P. palmivora	250	3.14*a	19.7 b	3.8 a	100	10
P. megakarya	250	3.19*a	1.7*c	0.6*b	50	0
P. citrophthora	500	2.30 b	35.5 a	4.5 a	100	50
P. palmivora	500	2.79*ab	15.6 b	4.1 a	100	20
P. megakarya	500	3.20*a	4.5*c	1.6 b	80	0
Control		3.60	0.0	0.0	0	0
Experiment 2						
P. citrophthora	100	2.90*a	23.9 a	3.8 a	100	20
P. palmivora	100	3.45*a	8.2 b	2.3 b	90	0
P. megakarya	100	3.62*a	0.5*c	0.2*c	20	0
P. citrophthora	250	2.55 b	30.1 a	4.5 a	100	50
P. palmivora	250	3.13*ab	15.7 b	3.9 a	100	30
P. megakarya	250	3.54*a	1.5*c	0.6*b	50	0

Table 3-14 Continued.

Species	ID	Root weight[v] (g)	# Colonies root syst[w]	Severity index[x]	% Infect[y]	% Mort[z]
P. citrophthora	500	2.33 b	48.3 a	4.5 a	100	50
P. palmivora	500	2.64 ab	17.4 b	4.4 a	100	40
P. megakarya	500	3.23*a	2.5*c	1.0 b	80	0
Control		3.65	0.0	0.0	0	0

[u]Both experiments were conducted at the University of Florida.
[v]Each value in this column is the average of root weights of 10 plants. Means followed by the same letter were not significantly different according to Duncan's multiple range test (P<0.01). Comparisons were valid within the same column only for means at each inoculum dosage. Means in each column followed by an asterisk were not different from the control according to Dunnett's test (P<0.01).
[w]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.
[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.
[y]Infection, expressed as the percentage of 20 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.
[z]Mortality is expressed as the percentage of 20 inoculated plants that had died 8 weeks after inoculation.

Table 3-15. Root weight, disease incidence, disease severity and percentages of infected plants and mortality 8 weeks after planting 5-week-old cacao seedlings of the cultivar ICS 1 in soil infested with different densities of chlamydospores of Phytophthora citrophthora, P. palmivora or P. megakarya per gram of soil.

Species	ID	Root[v] weight (g)	# Colonies root syst[w]	Severity index[x]	% Infect[y]	% Mort[z]
Experiment 1[u]						
P. citrophthora	100	2.29 c	23.0 a	4.1 a	100	20
P. palmivora	100	2.95*b	2.2 b	0.8*b	70	0
P. megakarya	100	3.58*a	0.4*b	0.3*b	30	0
P. citrophthora	250	2.20 b	24.3 a	4.4 a	100	40
P. palmivora	250	2.46*b	12.4 b	3.2 b	100	20
P. megakarya	250	3.52*a	1.8*c	0.7*c	40	0
P. citrophthora	500	2.12 b	24.8 a	4.5 a	100	50
P. palmivora	500	2.15 b	9.7 b	3.7 b	100	20
P. megakarya	500	3.60*a	3.0*c	1.0 c	70	0
Control		3.15	0.0	0.0	0	0
Experiment 2						
P. citrophthora	100	2.83 b	26.1 a	4.1 a	100	20
P. palmivora	100	2.99 b	3.0*b	1.2 b	70	0
P. megakarya	100	3.56*a	0.5*b	0.2*c	20	0
P. citrophthora	250	2.16 b	38.6 a	4.5 a	100	50
P. palmivora	250	2.53 b	12.3 b	3.3 b	100	20
P. megakarya	250	3.56*a	1.5*c	0.6*c	40	0

Table 3-15 Continued.

Species	ID	Root[v] weight (g)	# Colonies root syst[w]	Severity index[x]	% Infect[y]	% Mort[z]
P. citrophthora	500	2.09 b	36.2 a	4.5 a	100	50
P. palmivora	500	2.25 b	10.3 b	3.6 b	100	20
P. megakarya	500	3.53*a	3.2*c	1.2 c	80	0
Control		4.02	0.0	0.0	0	0

[u]Both experiments were conducted at the University of Florida.
[v]Each value in this column is the average of root weights of 10 plants. Means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.01$). Comparisons were valid within the same column only for means at each inoculum dosage. Means in each column followed by an asterisk were not different from the control according to Dunnett's test ($P<0.01$).
[w]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.
[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.
[y]Infection, expressed as the percentage of 20 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.
[z]Mortality is expressed as the percentage of 20 inoculated plants that had died 8 weeks after inoculation.

soil did not increase mortality over that observed at 250 chlamydospores per gram of soil.

The disease severity index was high for all cultivars exposed to 250 chlamydospores of P. citrophthora or P. palmivora per gram of soil; the index was also high for these species at 100 and 500 chlamydospores per gram of soil on SIC 23 and ICS 1, and at 100 chlamydospores per gram of soil on SIC 19 (Tables 3.12-3.15). Disease severity was greater with P. citrophthora than with P. palmivora at all inoculum levels with ICS 1 and at 100 chlamydospores per gram of soil with SIC 23. Trends similar to those observed for disease severity occurred for the number of colonies per root system of all of the cultivars with the three Phytophthora spp. Typically fewer than four infections of P. megakarya per root system were observed even at the highest inoculum levels. Although the numbers of colonies of P. megakarya generally increased with the higher inoculum levels, low disease incidence and severity occurred with this species for all cultivars as compared with P. citrophthora or P. palmivora. Root weights of all four cultivars were reduced by P. citrophthora at 250 or 500 chlamydospores per gram of soil when compared to the controls, and P. palmivora reduced root weights of plants of PA 121 at 250 chlamydospores per gram of soil and plants of ICS 1 at 500 chlamydospores per gram of soil.

More uniformity of results between the two experiments performed with each cultivar were observed for tests with chlamydospores than for those with zoospores. Greater numbers of colonies of P. citrophthora and P. palmivora grew from root systems of plants inoculated with chlamydospores in tests conducted in Florida than in Brazil. In tests conducted in Brazil, the severity index was higher for all species of Phytophthora with SIC 19 than with PA 121 (Tables 3.12 and 3.13).

Percentages of infection of all four cultivars were greater in soil infested with 250 oospores of P. capsici per gram of soil than in soil with 100 oospores per gram of soil (Tables 3.16-3.19). Similarly infection was generally greater with P. heveae on PA 121 and SIC 19 at the higher inoculum level in tests conducted in Brazil. The only mortality observed in these tests occurred with the high level of oospores of P. capsici and P. heveae in one experiment with SIC 19 (Table 3.17). Disease severity and numbers of colonies per root system were generally greater at 250 than at 100 oospores per gram of soil (T test at $P<0.05$). Reductions of root weight were generally observed only at 250 oospores of P. capsici or P. heveae per gram of soil, but an increase of root weight of infected plants of ICS 1, as compared to controls, was observed for both levels of inoculum of P. capsici (Table 3.19).

Table 3-16. Root weight, disease incidence, disease severity, and percentages of infected plants and mortality 8 weeks after planting 2-month-old cacao seedlings of the cultivar PA 121 in soil infested with different densities of oospores of Phytophthora capsici or P. heveae per gram of soil.

Species	ID	Root [v] weight (g)	# Colonies/ root syst.[w]	Severity index[x]	% Infect[y]	% Mort[z]
Experiment 1[u]						
P. capsici	100	2.71 a	0.2*a	0.2*a	20	0
P. heveae	100	2.85*a	1.8*a	0.6*a	60	0
P. capsici	250	2.65 a	1.3*b	0.6*b	60	0
P. heveae	250	2.78 a	3.6 a	1.2 a	70	0
Control		3.14	0.0	0.0	0	0
Experiment 2						
P. capsici	100	2.69*a	0.2*b	0.2*a	20	0
P. heveae	100	2.35*a	2.0 a	0.6 a	50	0
P. capsici	250	2.42*a	1.5*b	0.7*a	70	0
P. heveae	250	2.00 b	3.3 a	1.1 a	80	0
Control		2.65	0.0	0.0	0	0

[u]Both experiments were conducted at CEPLAC, Brazil.
[v]Each value in this column is the average of root weights of 10 plants. Means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.01$). Comparisons were valid within the same column only for means at each inoculum dosage. Means in each column followed by an asterisk were not different from the control according to Dunnett's test ($P<0.01$).

Table 3-16 Continued.

[w]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.
[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.
[y]Infection, expressed as the percentage of 10 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.
[z]Mortality is expressed as the percentage of 10 inoculated plants that died 8 weeks after inoculation.

Table 3-17. Root weight, disease incidence, disease severity, and percentages of infected plants and mortality 8 weeks after planting 2-month-old cacao seedlings of the cultivar SIC 19 in soil infested with different densities of oospores of Phytophthora capsici or P. heveae per gram of soil.

Species	ID	Root weight (g)[v]	# Colonies/ root syst.[w]	Severity index[x]	% Infect[y]	% Mort[z]
Experiment 1[u]						
P. capsici	100	1.79*a	0.9*a	0.5*a	50	0
P. heveae	100	1.80*a	1.0*a	0.4*a	40	0
P. capsici	250	1.12 a	8.5 a	3.6 a	100	40
P. heveae	250	1.17 a	9.5 a	4.0 a	100	50
Control		1.91	0.0	0.0	0	0
Experiment 2						
P. capsici	100	1.94 a	2.5 b	0.8*a	60	0
P. heveae	100	1.59*a	5.1 a	1.6 a	100	0
P. capsici	250	1.57*a	3.8 b	1.3 b	80	0
P. heveae	250	1.15*b	7.2 a	3.1 a	100	0
Control		1.47	0.0	0.0	0	0

[u]Both experiments were conducted at CEPLAC, Brazil.
[v]Each value in this column is the average of root weights of 10 plants. Means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.01$). Comparisons were valid within the same column only for means at each inoculum dosage. Means in each column followed by an asterisk were not different from the control according to Dunnett's test ($P<0.01$).

Table 3-17 Continued.

[W]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.
[X]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.
[Y]Infection, expressed as the percentage of 10 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.
[Z]Mortality is expressed as the percentage of 10 inoculated plants that died 8 weeks after inoculation.

Table 3-18. Root weight, disease incidence, disease severity, and percentages of infected plants and mortality 8 weeks after planting 2-month-old cacao seedlings of the cultivar SIC 23 in soil infested with different densities of oospores of Phytophthora capsici per gram of soil.

Test[u]	ID	Root weight[v] (g)	# Colonies/ root syst.[w]	Severity index[x]	% Infect[y]	% Mort[z]
1	100	3.71a	0.4b	0.2b	20	0
	250	2.94b	4.5a	1.6a	90	0
	Control	3.63a	0.0b	0.0b	0	0
2	100	3.55a	0.4b	0.2b	20	0
	250	2.91b	3.9a	1.3a	80	0
	Control	3.45a	0.0b	0.0b	0	0

[u]Both experiments were conducted at the University of Florida.
[v]Each value in this column is the average of root weights of 10 plants. Means followed by the same letter were not significantly different according to Duncan's multiple range test (P<0.01). Comparisons were valid within the same column only for means at each inoculum dosage in an individual experiment. Means in each column followed by an asterisk were not different from the control according to Dunnett's test (P<0.01).
[w]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.
[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.
[y]Infection, expressed as the percentage of 10 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.
[z]Mortality is expressed as the percentage of 10 inoculated plants that died 8 weeks after inoculation.

Table 3-19. Root weight, disease incidence, disease severity, and percentages of infected plants and mortality 8 weeks after planting 2-month-old cacao seedlings of the cultivar ICS 1 in soil infested with different densities of oospores of Phytophthora capsici per gram of soil.

Tests[u]	ID	Root weight[v] (g)	# Colonies/ root syst.[w]	Severity index[x]	% Infect[y]	% Mort[z]
1	100	3.25a	0.5b	0.2b	20	0
	250	3.43a	5.8a	1.6a	90	0
	Control	2.96b	0.0b	0.0b	0	0
2	100	3.31a	0.7b	0.2b	20	0
	250	3.40a	6.6a	2.2a	90	0
	Control	3.16b	0.0b	0.0b	0	0

[u]Both experiments were conducted at the University of Florida.
[v]Each value in this column is the average of root weights of 10 plants. Means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.01$). Comparisons were valid within the same column only for means at each inoculum dosage in an individual experiment. Means in each column followed by an asterisk were not different from the control according to Dunnett's test ($P=0.01$).
[w]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.
[x]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap root; 5=plant moribund (wilted) or dead.
[y]Infection, expressed as the percentage of 10 inoculated plants that were infected 8 weeks after inoculation, was determined by plating roots on a selective medium.
[z]Mortality is expressed as the percentage of 10 inoculated plants that died 8 weeks after inoculation.

Discussion

Phytophthora capsici, *P. citrophthora*, *P. heveae*, *P. megakarya*, and *P. palmivora* all infected roots of 5-to 8-week-old cacao seedlings. All forms of inoculum evaluated resulted in infection of roots of the five cultivars of cacao tested. In the present study disease severity and numbers of infection generally were greatest at the highest inoculum concentrations. However, host responses to the pathogen varied with the species of *Phytophthora* and cultivar of the host tested.

All root systems of the five cultivars tested were infected with *P. citrophthora* at 10^6 zoospores per plant and 250 chlamydospores per gram of soil; up to 70 to 80% of the seedlings were killed at these inoculum levels. High levels of infection and some mortality were also observed with plants exposed to 5×10^5 zoospores per plant and 100 chlamydospores per gram of soil. The ability of *P. citrophthora* to attack other parts of the cacao plant was discussed in Chapter 2. The present results are in accordance with the findings of Kellam and Zentmyer (70) and reinforce their suggestion that *P. citrophthora* has the potential to be a severe pathogen of cacao roots.

Phytophthora palmivora was infective at all levels of zoospores and chlamydospores tested. However, wilting and mortality of plants were only observed at 2×10^5 zoospores

or more per plant. At 10^6 zoospores per plant, 100% infection of all cultivars occurred and up to 50% of the plants were killed. One hundred percent infection and 20 to 40% mortality were detected in plants of the four cultivars when inoculated with 250 or 500 chlamydospores of P. palmivora per gram of soil. At the levels of inoculum tested, P. palmivora and P. citrophthora caused similar damage to all of the cultivars. Thus, the inoculum levels for screening tests for disease resistance, at least with these two species, do not have to be greater than 5×10^5 zoospores per plant or 100 chlamydospores per gram of soil.

Phytophthora palmivora has been isolated from roots of moribund seedlings and rootstocks in nurseries in Brazil (Luz and Mitchell, unpublished) and from poorly developed root systems of cacao seedlings in Ghana (145). In Nigeria, P. palmivora was occasionally isolated from feeder roots of mature cacao trees (148). This species has also been isolated from roots of other host plants, such as coconut palms (58) and papaya (72, 135). The present study demonstrates that this fungus can readily infect roots of several cacao cultivars and kill seedlings.

Even at the highest levels of inoculum of P. capsici tested, 100% infection of plants was obtained only in some experiments with some of the cultivars. Fragments of mycelium of P. capsici used as inoculum did not cause infection in plants of SIC 23 and ICS 1. Damage caused by

P. capsici to the root systems of cacao plants exposed to zoospores or oospores was generally less severe than disease caused by P. citrophthora or P. palmivora. If mortality of plants exposed to zoospores or oospores of P. capsici occurred at all, it was typically at low percentages (10-40%) and was inconsistent over repeated tests. However, when periodically flooded, 20% of the SIC 19 seedlings planted in soil containing 250 oospores per gram of soil were killed. Under nonflooded conditions, there is the possibility that 8 weeks is not time enough for oospores of P. capsici to germinate, infect, and kill 5- to 8-week-old plants, even at the highest levels tested in these experiments. Kellam and Zentmyer (70) obtained 40% infection and no mortality of cacao seedlings grown after 8 weeks in soil infested with 200 oospores of P. capsici per gram of soil. As in the present study, Kellam and Zentmyer (70) only tested oospores from one cross. According to the findings of Bowers (15), variation in virulence of isolates from oospores produced in different crosses between compatibility types of P. capsici may occur; however, experimentation needs to be expanded to take into consideration variation in constitutive dormancy in oospores from different crosses. This suggests that oospore inocula from several crosses within a population of isolates of P. capsici from cacao should be used in future experiments to define the

role of oospores in the ecology and epidemiology of P. capsici in cacao.

Inoculation of seedlings of PA 121 and SIC 19 with zoospores of P. heveae was performed once and the experiment needs to be repeated to confirm the results (Tables 3.8 and 3.9). However, 100% infection, but no mortality, was obtained with plants of SIC 19 at 5×10^5 zoospores per plant, and with plants of PA 121 or SIC 19 at 10^6 zoospores per plant. More severe damage to cacao seedlings generally occurred with P. heveae than with P. capsici at the highest level of oospores tested. Fifty percent of the plants of SIC 19 inoculated with 250 oospores of P. heveae under non-flooded conditions died while 80% of the flooded plants were killed at the same inoculum concentration (Tables 3.7 and 3.17). Oospores were observed in roots of dead and infected plants, which indicates the possibility of these spores functioning as inoculum for further infections. The role of P. heveae as a cacao root pathogen needs to be elucidated by additional studies, but this fungus appears to have the potential to cause damage to the crop, especially under flooded conditions.

Phytophthora megakarya was the only species that did not infect cacao roots at the lowest zoospore concentration tested (10^5 zoospores per plant), but only low levels of infection were generally observed with the other species tested with cultivars SIC 19 and SIAL 325 at this inoculum

level (Tables 3.10 and 3.11). Phytophthora megakarya did not kill plants at any inoculum level tested with either chlamydospores or zoospores. High levels of infection (80 and 100%) were obtained only with 500 chlamydospores per gram of soil and 10^6 zoospores per plant, respectively. The highest severity index values (1.8 and 1.9) obtained with this species were with cultivars SIC 19 and SIAL 325, respectively, after inoculation with 10^6 zoospores per plant (Tables 3.10 and 3.11). Consequently, it appears that P. megakarya only infected root tips of feeder roots, as indicated by the severity index, and, even at high inoculum dosages, seldom more than seven colonies developed from root systems of infected seedlings. Generally, P. megakarya caused less damage than the other four Phytophthora spp. to the root systems of all of the cultivars tested.

Based on the findings of Ward and Griffin (148) in Nigeria, Gregory (53) and Gregory et al. (54) suggested that P. megakarya may be an active cacao root pathogen that attacks tips of feeder roots of mature cacao trees. Phytophthora megakarya is the main pathogen on cacao in Nigeria, and may function as a damaging root pathogen there. Conversely, it is possible that the association of P. megakarya with cacao roots only serves as a way to survive the dry season, when no pods are available for infection. As stated in Chapter 2, this species did not cause severe damage to pods, leaves, seedlings, and excised roots of

cacao in the present studies. Future studies on the relationship of inoculum density of P. megakarya to disease incidence in cacao should be conducted with susceptible cultivars from Africa under environmental conditions known to be conducive to disease.

The disease severity index proved to be a useful parameter in quantitative comparisons of infection and disease occurring at different inoculum levels of the Phytophthora spp. with all of the cultivars tested. This parameter incorporated the values of number of colonies per root system, disease incidence, and mortality. It proved to have less variation (smaller Coefficient of Variation) than that observed for number of colonies per root system. Number of colonies per root system, percentage of infection, and mortality are important parameters for the evaluation of cacao root disease, but none of them alone appears to provide adequate estimations of disease. The number of colonies did not always increase with increase of disease severity. Dead plants of SIC 19 infected with zoospores of P. citrophthora had smaller numbers of colonies than other plants of the same cultivar that were infected and showed no external symptoms.

The location of the sites of infection is important for the assessment of disease severity caused by Phytophthora spp. on cacao roots. Just one lesion on the tap root can cause more damage to the plant than can several infections

on first-order roots. The disease severity index was tentatively employed to elucidate importance and variation in root infection and subsequent disease. Results indicate that this parameter of disease evaluation can be useful in quantitative comparisons of resistance of cacao cultivars to root infection. Indices of disease severity were also used with success to evaluate Phytophthora root rot of alfalfa (13, 52).

Reductions of root weight were great only with high levels of disease caused by P. citrophthora or P. palmivora. Correlations of either root weight or root length of infected plants to numbers of colonies per root system were not significant ($P<0.05$) (Luz and Mitchell, unpublished). This may have been due, at least partially, to the extreme variability among plants of each cultivar. In most cases root weights of infected plants did not differ from those of control plants, and in some cases, as for example with cultivar ICS 1, infected plants weighed more than the controls. Stimulated root growth may be a response of this cultivar to infection. Some cultivars appear to have the tendency to produce new primary roots to compensate for the loss of other roots to disease caused by Phytophthora spp.

The inoculum densities used in this study were much higher than the levels of zoospores, chlamydospores, or oospores typically employed in quantitative epidemiological studies with Phytophthora spp. Mitchell and Kannwischer-

Mitchell (103) reviewed the literature on relationships between inoculum densities of Phytophthora spp. and disease incidences in various hosts, and presented several examples of infection and mortality with low levels of inoculum. For example, only 42 zoospores or 8.6 chlamydospores (0.13 chlamydospores per gram of soil) of P. parasitica var. nicotianae (Breda de Haan) Tucker per tobacco plant induced 50% infection, and most of the infected plants died within 14 to 28 days after inoculation (64). Similarly, 40 and 60% mortality of pepper seedlings were observed with inoculum densities of 10 and 15 oospores of P. capsici per gram of soil, respectively (15). However, as has been found with some other pythiaceous fungi (103), disease and mortality in cacao may occur following infection with the pathogens used in this study only when roots are exposed to high levels of inoculum, or when there are secondary cycles of inoculum production and infections after limited initial infection of host roots. Mitchell (102), for example, reported that 250 to 280 zoospores per plant of P. cryptogea, Pythium ostracoides, or Pythium aphanidermatum caused 50% infection on watercress, cotton, and tomato, respectively, but 10^4 zoospores per plant were required to reach 50% mortality of these plants. Since this study emphasized comparisons of several species using at least two cultivars in each test, only two or three inoculum levels, which were selected to be high enough to cause mortality in at least some of the

plants, could be used. Perhaps much higher levels of inoculum than those employed in this study are required for high incidences of disease. Nevertheless, in the pathosystems with Phytophthora spp. and cacao, even when root damage and plant mortality are minimal after root infection, the importance of infection sites and the production of secondary inoculum should be considered in future studies.

Flooding had little effect on disease caused by zoospores of P. capsici or by zoospores or chlamydospores of P. citrophthora, P. megakarya, and P. palmivora. In contrast, periodically flooded plants growing in soil infested with oospores of P. capsici or P. heveae were more severely damaged than plants growing under freely drained conditions. These differential responses with the different spore forms to flooding are difficult to explain. The use of flooding at the time of inoculation with zoospores resulted in high levels of infected seedlings of different hosts by many species of Phytophthora and Pythium (17, 64, 102, 103, 104, 134). However, soil flooding before inoculation with zoospores did not predispose cherries to root disease caused by P. cryptogea (154) or Fraser fir seedlings to root rot caused by P. cinnamomi (71). In experiments with different cherry and walnut rootstocks and rooted cuttings of red raspberry, the level of disease that developed as the duration of periodic flooding increased depended on the

particular combination of rootstocks and Phytophthora spp. involved; some combinations required no flooding for severe root and crown rot to develop, whereas other combinations required prolonged flooding for severe disease to occur (40, 93, 94, 154). With apple seedlings, periodic flooding of soil favored disease development by P. cactorum and P. cambivora; in contrast, flooding was not essential for development of root and crown rot caused by P. cryptogea (19).

Bowers (15) observed a dramatic increase in mortality of peppers grown under defined soil water potential in Buchner funnels when soil infested with oospores of P. capsici was periodically flooded. However, very low or no plant mortality was observed in treatments with either periodic flooding or routine watering in three separate experiments conducted in pots under greenhouse conditions. The soil and the inoculum were the same as those used in successful experiments with Buchner funnels. Bowers (15) considered the different responses to be due to "some unknown factors" (pg. 58). In the present experiment, periodic flooding of oospore-infested soil may have stimulated root leakage and subsequent oospore germination, formation of secondary inoculum, and the increase of the number of infections in the root systems of susceptible cacao seedlings. Further studies need to be conducted with cacao grown under controlled water potentials with periodic

flooding after inoculations with Phytophthora spp. to determine if disease incidence is increased or if inoculum is more efficient under conditions such as those described by Bowers (15).

As discussed by Kannwischer and Mitchell (64), it is desirable to use raw soil in studies dealing with the epidemiology of root diseases. They found, however, that by allowing recolonization of autoclaved soil by airborne microorganisms for 30 days before infesting the soil, similar inoculum densities were required for infection of tobacco plants by P. parasitica var. nicotianae in both autoclaved and raw soil. Similar numbers of individual roots per root system of tobacco were also infected by this fungus in autoclaved, recolonized soil or raw soil (44). In the present study the numbers of colonies of P. palmivora growing from the root systems were always greater in pasteurized than in raw soil. This greater infection response in the pasteurized than in the raw soil and the presence of indigenous pythiaceous fungi in raw soil led to the use of pasteurized soil in the subsequent experiments. The lower levels of root colonization in raw soil than in pasteurized soil may have been due to competition between added inoculum and inoculum of indigenous species of pythiaceous fungi for available infection sites on cacao roots, to the activities of antagonists in the raw soil, or general nutrient competition with other non-specific soil saprophytes.

Considerable variations in disease response occurred among replications of individual treatments and between experiments conducted under similar conditions when the same soil and same procedure for inoculum production were used. The following factors may have been at least partially responsible for these variations: 1) the high level genetic heterogeneity observed in cacao (discussed in Chapter 2), 2) seedlings used for root inoculations were obtained from pods from open-pollinated plants, 3) pods were obtained from different trees and different areas because of the numbers of seedlings required for these tests, and 4) the age of the seedlings. Variations were observed among experiments with cultivar SIC 19 conducted in both Florida and Brazil. It is possible that these variations were due primarily to different experimental conditions in these locations. For instance, plants in Florida were grown in sandy soil while clay loamy soil was used in Brazil. Although the experiments were conducted under greenhouse conditions at both locations, biotic and environmental factors vary under these conditions and can affect disease development (13). Bowers (15) observed variation in disease responses of pepper seedlings inoculated with zoospores of P. capsici and suggested that the overall germinability or efficiency of zoospore or oospore inoculum could also vary among

experiments, and, thus, could contribute to variation in these types of studies.

Despite variations in plant responses, such as root weights, similar trends in levels of infection generally were observed with each species of Phytophthora in experiments conducted in Florida and Brazil. Also, the cultivars had similar reactions to each species in spite of the different kinds of soil used and the different environmental conditions at the two locations. Cultivars SIC 19, SIC 23, ICS 1, and SIAL 325 were all less tolerant to root infection by P. citrophthora and P. palmivora, than by P. megakarya and, in the few cases in which it was tested, P. heveae. Responses of these cultivars to P. capsici were variable, but tolerance to this pathogen appeared to be intermediate to that of P. megakarya and P. citrophthora or P. palmivora. Cultivar PA 121 showed some tolerance to P. citrophthora and P. palmivora when the lower levels of zoospores or chlamydospores were used, and it was tolerant to P. capsici, P. heveae, and P. megakarya even at the highest inoculum level. Both susceptible and tolerant cacao seedlings were infected in soil infested with zoospores, chlamydospores and oospores of Phytophthora spp., but seedlings of the tolerant cultivars rarely died as a result of infection.

The present results show that all five Phytophthora spp. have the potential to infect cacao root systems and indicate that, as was hypothesized by Gregory (53, 54),

cacao root infection may play a major role in the epidemiology of black pod under field conditions. Although the plants may not show any external symptoms, invaded roots function as reservoirs of inoculum that may have subsequent roles in disease of the aerial parts of the cacao plant. With the exception of the isolate of P. heveae, the isolates of Phytophthora spp. tested in this study were obtained from infected pods. To avoid the possibility of isolate specialization in future studies, it will be important to test the pathogenicity of isolates obtained from naturally infected cacao roots.

Chapter 4
EVALUATION OF TOLERANCE OF CACAO CULTIVARS TO ROOT INFECTION BY PHYTOPHTHORA SPP.

Introduction

The use of resistant or tolerant cultivars is often one of the most effective and economical means of controlling plant diseases. Over the last 30 years the search for cacao cultivars resistant to black pod disease resulted in the selection of many cultivars in different parts of the world that have some resistance or tolerance to pod infection by Phytophthora spp. (29, 43, 77, 121, 122, 130, 131). Inconsistent responses of some cultivars to disease in different areas of world have been attributed to genetic variation in the host, diversities of environmental conditions, presence of different races or variants of the pathogen, and to the diversity of test methods used by workers in different countries to evaluate resistance (76). The screening tests for resistance to black pod included evaluation of disease in pods, leaves, stems, seedling roots, rooted cuttings, and seeds inoculated with or without wounding (14). Mycelium, zoospores, or fragments of infected tissue typically have been used for inoculum. The discovery that more than one

species of Phytophthora cause black pod may explain some of the discrepancies reported in the responses of cacao cultivars to infection in different countries. Thus, there is a continuing need to search for plant material, resistant or tolerant to species of the pathogen in the areas in which the tests will be conducted and for standardized methodology for screening for resistance (81).

Although the main objective of screening tests for resistance to black pod has been to find cultivars resistant to pod infection or disease by Phytophthora spp., the need to devise a quick method for screening large quantities of material led to the use of young seedlings, seeds, leaves or other organs of cacao in studies of black pod resistance (14). Turner and Asomaning (145) suggested that a comparative study of selections of cacao for resistance to root disease caused by P. palmivora might indicate inherent resistance to the pod rot disease. Intensive work was then carried out in Ghana to evaluate resistance of young clones and seedlings of cacao to root infection by P. palmivora (10, 11, 146, 149). Cacao selections were considered resistant or susceptible based on a quantitative analysis of plant growth after root infection and compared to observations in the field for black pod. Tests with clones revealed similarities in resistance to root infection and resistance to pod infection by P. palmivora (8, 10, 146). Zentmyer et al. (165) also described a method of evaluating

infection of roots of cacao seedlings; seedlings of cultivars considered to be susceptible, resistant or of intermediate resistance were grown in aerated modified Hoagland's nutrient solution infested with pieces of colonies of P. palmivora in V-8 agar. Estimations of the percentages of root systems infected were used to evaluate the performance of cultivars, and a positive correlation between root infection and pod reaction was observed.

Based on the reinterpretation of the epidemiological studies of the cacao research group in Nigeria by Gregory et al. (54), which emphasized the possible role of cacao roots in black pod epidemics, Zentmyer (161) suggested that a screening test for root resistance to Phytophthora spp. would be of great value for disease control in the field. The Cacao Research Center (CEPEC) at CEPLAC, Itabuna, Bahia, Brazil, has conducted screening tests of cacao cultivars for black pod resistance for several years, and various cultivars have been selected with tolerance or resistance to Phytophthora spp. (80, 81, 83, 88, 98, 121, 122, 124, 125, 127, 129). Emphasis in this program has been placed on the evaluation of cultivars from wild selections that originated from the upper Amazon when challenged with isolates of P. capsici, P. citrophthora, and P. palmivora. Cultivars that were tested and rated as resistant in other countries, or in Brazil before it was known that more than one species of Phytophthora caused black pod disease, have also been

included in these tests. More than 80 cultivars have been tested, and some of these have resistance or tolerance to all three Phytophthora spp. and will be incorporated into breeding programs for disease resistance. If root disease caused by Phytophthora spp. is demonstrated to be damaging to cacao production, it will be important to determine if cultivars that are resistant, tolerant, or susceptible to pod infection respond similarly to root disease caused by each species of the pathogen. The objectives of this study were to evaluate responses of selected cacao cultivars to root infection by P. capsici, P. citrophthora, P. heveae, P. megakarya, and P. palmivora; to develop methods of quantitatively comparing the levels of tolerance or resistance in various cultivars; and to compare the responses of cultivars to root and stem inoculations.

Materials and Methods

Greenhouse studies were conducted at the University of Florida, in Gainesville, Florida and at CEPLAC in Itabuna, Bahia, Brazil to determine the responses of cacao cultivars to root infection by Phytophthora spp. Plants were grown as described in chapters 2 and 3. Isolates P23 of P. capsici, P62 of P. citrophthora, P208 of P. heveae, Etoun or P614 of P. megakarya and P26 of P. palmivora, all described in Chapter 2, were used to produce zoospores and chlamydospores

for inoculations of cacao seedlings. Oospores of P. capsici were obtained from crosses between P23 and P1043, of the A^1 and A^2 compatibility types, respectively. The different forms of inoculum of each species were obtained as described in Chapters 2 and 3.

Zoospore inoculations were performed as previously described (Chapter 3) and the final concentration of zoospores of each of the five species was 5×10^5 zoospores per plant in all of the inoculation tests performed. Plants of all cultivars were grown at CEPLAC in individual plastic bags with drainage holes, and each bag contained 750 g of steam-pasteurized clay loamy soil.

Responses of cacao cultivars to root infection were evaluated in Florida by using soil infested with chlamydospores of P. citrophthora, P. megakarya, and P. palmivora, or oospores of P. capsici using the infested-soil-layer technique described by Mitchell (101) and Kannwischer and Mitchell (64). Four hundred grams of steam-pasteurized sandy soil infested with 100 chlamydospores or oospores per gram of soil, were layered over 400 g of steam-pasteurized non-infested soil in a 10.5-cm-diameter plastic pot and covered with 200 g of steam-pasteurized, non-infested soil. One 8-week-old cacao seedling was transplanted into each pot.

Plants grown in soil infested with chlamydospores were maintained in a greenhouse in Florida at 24-27 C and were

watered daily from the bottom by filling saucers under the pots. Plants inoculated with zoospores were maintained under similar conditions and watered by filling the saucers under the plastic bags. The percentages of zoospore germination, and the initial detectable inoculum density in each lot of soil infested with chlamydospores or oospores were assayed as described previously (Chapter 3).

In the experiments conducted using zoospores as inoculum, 15 cacao seedlings, obtained from open pollinated seeds of each of 15 cultivars from CEPLAC, Brazil, were inoculated with each of the five Phytophthora spp. The experiment was repeated one time. The 15 cacao cultivars used in this experiment have been reported to have different levels of resistance to pod infection by P. capsici, P. citrophthora, and P. palmivora, and their responses to these species and their origins are listed in Table 4.1. The chlamydospore or oospore inoculations were performed with the following eight cultivars: CA 3, CAS 2, EET 228, PA 121, PA 169, SIAL 325, SIC 17, and SIC 19. A minimum of seven seedlings of each of the cultivars was inoculated with each species in each of two consecutive tests. Non-inoculated seedlings, equal in number to inoculated seedlings of each of the cultivars tested, were included in all of the tests as controls.

Table 4-1. Origin of 15 cultivars of cacao and their ratings for resistance to disease caused by Phytophthora spp. in various screening tests.

Cultivar	Origin	Resistance to Phytophthora spp.[x]	Country/year of evaluation	Reference
BE 5	Belem, Para, Brazil	T/ P. citrophthora; S/P. palmivora and P. capsici	Brazil, 1985	Luz, et al.[y]
CA 3	Carreiro, Amazonas, Brazil	T/ P. citrophthora, P. palmivora and P. capsici	Brazil, 1985	Luz, et al.
CAS 2	Santarem, Para, Brazil	R/ P. capsici ?	Brazil, 1971	(127)
		T/ P. citrophthora; R/ P. capsici and P. palmivora	Brazil, 1985	Luz, et al.
EEG 8	Goitacazes, Espirito Santo, Brazil	R/ P. capsici ?	Brazil, 1969	(125)
		S/ P. citrophthora; T/ P. capsici and P. palmivora	Brazil, 1985	Luz, et al.
EET 228	Pichilingue, Ecuador	S/ P. citrophthora; T/ P. palmivora and P. capsici	Brazil, 1985	Luz, et al.
EET 59	Pichilingue, Ecuador	S/ P. citrophthora; T/ P. palmivora and P. capsici	C. R.[z], 1978	(77)
			Brazil, 1983	(80)
MA 15	Manaus, Amazonas, Brazil	T/ P. citrophthora, P. palmivora and P. capsici	Brazil, 1987	(129)
PA 30	Parinari, Peru	R/ P. capsici ?	Brazil, 1973	(124)
		T/ P. citrophthora; R/ P. palmivora and P. capsici	Brazil, 1985	Luz, et al.

Table 4-1 continued.

Cultivar	Origin	Resistance to *Phytophthora* spp.[x]	Country/Year of evaluation	Reference
PA 121	Parinari, Peru	R/ *P. capsici* ?	Brazil, 1974	(122)
		T/ *P. citrophthora*; R/ *P. palmivora* and *P. capsici*	Brazil, 1985	Luz, et al.
PA 169	Parinari, Peru	R/ *P. palmivora* ?	Jamaica, 1975	(59)
		T/ *P. citrophthora*; R/ *P. palmivora* and *P. capsici*	Brazil, 1985	Luz, et al.
SIAL 325	Jucari, Bahia, Brazil	S/ *P. citrophthora*, *P. palmivora* and *P. capsici*	Brazil, 1983	(80)
SIC 17	Urucuca, Bahia, Brazil	S/ *P. citrophthora*, *P. palmivora* and *P. capsici*	Brazil, 1985	Luz, et al.
SIC 19	Urucuca, Bahia, Brazil	S/ *P. citrophthora*, *P. palmivora* and *P. capsici*	Brazil, 1985	Luz, et al.
SIC 23	Urucuca, Bahia, Brazil	S/ *P. citrophthora*, *P. palmivora* and *P. capsici*	Brazil, 1985	Luz, et al.
SIC 806	Urucuca, Bahia, Brazil	R/ *P. capsici* ?	Brazil, 1972	(124)
		S/ *P. citrophthora*; T/ *P. palmivora*; R/ *P. capsici*	Brazil, 1987	(129)

[x]T=Tolerant; R=some level of resistance; S=susceptible.
[y]Unpublished work by Luz, E.D.M.N., M.M. Yamada, and S.D.V.M. Silva.
[z]C.R.=Costa Rica.

The numbers of wilted or dead plants in all experiments were recorded. Procedures used to evaluate the disease with each cultivar and each species were described in Chapter 3. Disease severity in root systems of all of the cultivars was assessed 8 weeks after inoculation. The scale for the disease severity index was: 0, no infection; 1, one to four first-order roots infected; 2, five to seven first-order roots infected; 3, one to two second-order roots infected or more than seven first-order roots infected; 4, more than two second-order roots infected, one or more colonies on tap root, or one or more cankers on tap root; and 5, plant moribund (wilted) or dead. The soil populations of each species of Phytophthora in each cultivar were analyzed at harvest in experiments using zoospore infestations with the soil-dilution plating technique of Kannwischer and Mitchell (64). A 10-gram sample of soil was collected from the root zone of each of 10 plants per cultivar and suspended in 90 ml of 0.25% water agar. A 1-ml sample of soil suspension was spread over each of 10 petri plates containing PARPH (Chapter 2). After 2 or 3 days of incubation under laboratory conditions in the dark, plates were rinsed free of soil and colonies were counted. The dry weight of a 1-ml sample from each soil dilution was also determined. Populations, expressed as propagules per gram of dry soil (ppg), were determined by dividing the average number of colonies per plate of each of the 10 samples of soil from each culti-

var by the weight of the dried, 1-ml sample of the suspension of soil in water agar. A sample of soil from the control plants of each cultivar was also assayed.

A completely randomized experimental design was used in the inoculation experiments. The effect of each species of Phytophthora on root weight, number of colonies per root system, disease severity index, and number of propagules per gram of soil of all the cultivars was analyzed with the GLM procedure in SAS (Statistical Analysis System, SAS Institute Inc., Cary, NC). Means were compared statistically with Duncan's multiple range test.

Stem inoculations were performed in Florida with 6-month-old seedlings of the following nine cultivars listed in Table 4-1: CA 3, CAS 2, EET 228, PA 121, PA 169, SIAL 325, SIC 17, SIC 19, and SIC 23. The wound inoculation method was the same as that described in Chapter 2 (78, 165). Each of 10 cacao seedlings of each cultivar was inoculated with a 4-mm mycelial disk from a 4-day-old, carrot agar culture of P. capsici, P. citrophthora, P. megakarya or P. palmivora. The areas of lesions were evaluated 18 days after inoculation using the micro-comp data system described in Chapter 2. The data were analyzed as a two-factor factorial, completely randomized, block design, and the average areas of lesions on cultivars were compared for each of the species of Phytophthora tested using Duncan's multiple range test.

Results

High levels of infection of all cultivars evaluated occurred after stem inoculations or exposure to soil infested with high levels of zoospores or chlamydospores of the five species of Phytophthora (Tables 4-2 to 4-9 and Table 4-11). However, mortality and root weight losses generally were not severe, and the disease severity index indicated that, except with the least tolerant cultivars inoculated with P. citrophthora or P. palmivora, infection was generally limited to first- and second-order roots. Thus, all of the cultivars demonstrated some level of tolerance to root disease caused by Phytophthora spp.

Germination of zoospores of all Phytophthora spp. used for inoculations ranged from 84 to 95% after 24 hours of incubation on water agar (Table A-1, Appendix). Results of each replicated experiment for each species of Phytophthora, except P. heveae, were similar, and, consequently, the data from the two experiments were combined for statistical analysis. Due to a labor strike in Brazil, plants from the repeated test with P. heveae could not be harvested on time and were severely damaged by adverse environmental conditions; thus, the data from this experiment were not included

Table 4-2. Disease severity index, percentages of infection and mortality, colonies per root system, soil population, and root weight 8 weeks after planting 8-week-old cacao seedlings of 15 cultivars in soil infested with 5 x 10^5 zoospores of Phytophthora citrophthora per plant.

Cultivar	Severity index[t]	Infection (%)[u]	Mortality (%)[v]	# Colonies/ root system[w]	# Propagules/ g soil[x]	Root wt.[y] (g)	Root wt. loss[z] (g)
PA 30	2.1 a	93	0	6.5 a	0.0 a	3.08	0.09*
MA 15	2.5 b	100	0	8.4 abc	666.3 bcde	2.39	-0.33*
BE 5	2.6 bc	100	0	10.8 c	245.5 ab	2.91	0.07*
CA 3	2.7 bcd	100	0	9.4 abc	296.7 abc	2.11	-0.76
PA 169	2.7 bcd	100	7	7.7 ab	584.1 bcd	2.25	-0.81
PA 121	2.9 bcd	100	0	10.3 bc	586.1 bcd	3.11	-0.14*
CAS 2	2.9 bcd	100	0	14.1 de	1056.3 e	2.84	0.31*
SIC 806	2.9 bcd	100	0	10.2 bc	851.1 de	2.10	-0.43
EET 59	3.0 cde	100	0	16.9 e	461.0 bcd	3.11	-0.05*
EEG 8	3.1 de	100	0	15.1 e	543.4 bcd	2.79	-0.02*
EET 228	3.4 ef	100	20	13.9 de	276.4 abc	6.63	-0.01*
SIC 17	3.7 fg	100	0	14.1 de	2697.4 fg	1.82	-0.44
SIAL 325	3.7 fg	100	7	14.5 e	697.8 cde	2.34	-0.27*

Table 4-2 continued.

Cultivar	Severity index[t]	Infection (%)[u]	Mortality (%)[v]	# Colonies/ root system[w]	# Propagules/ g soil[x]	Root wt.[y] (g)	Root wt. loss[z] (g)
SIC 23	3.9 g	100	13	9.3 abc	2953.6 g	1.80	-0.43
SIC 19	3.9 g	100	33	11.3 cd	2462.6 f	1.63	-0.52

[t]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap roots; 5=plant moribund (wilted) or dead. Means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.05$); comparisons were valid within the same column.

[u]Infection is expressed as the percentage of 30 inoculated plants that were infected 8 weeks after inoculation as determined by plating roots on a selective medium.

[v]Mortality is expressed as the percentage of 30 inoculated plants that had died 8 weeks after inoculation.

[w]Average of total number of colonies obtained per root system as determined by plating on a roots on a selective medium.

[x]Average number of propagules recovered from 10 soil samples from each of 30 plants per cultivar 8 weeks after infestation (1 ml of a dilution of each sample was distributed on each of 10 plates of a selective medium).

[y]Average root weight of 30 plants.

[z]Difference between average root weights of the control and inoculated plants. Positive values represent greater root weights of infected plants than of control plants, while negative values indicate lower root weights of infected plants as compared to control plants. Values followed by an asterisk were not significantly different from the noninoculated control according to Dunnett's test ($P<0.05$).

Table 4-3. Disease severity index, percentages of infection and mortality, colonies per root system, soil population, and root weight 8 weeks after planting 8-week-old cacao seedlings of 15 cultivars in soil infested with 5 x 10^5 zoospores of Phytophthora palmivora per plant.

Cultivar	Severity index[t]	Infection (%)[u]	Mortality (%)[v]	# Colonies/ root system[w]	# Propagules/ g soil[x]	Root wt.[y] (g)	Root wt. loss[z] (g)
CA 3	1.4 a	94	0	4.0 a	71.5 a	1.82	-1.05
PA 30	1.9 ab	80	0	7.8 b	204.7 ab	2.73	-0.02*
MA 15	2.1 bc	94	0	7.7 b	947.8 d	1.81	-0.91
PA 169	2.2 bc	94	0	8.7 b	951.6 d	2.35	-0.71
EEG 8	2.4 bcd	94	0	7.7 b	1682.1 e	2.45	-0.06*
PA 121	2.5 bcd	100	0	9.1 b	245.5 ab	2.82	-0.42*
EET 59	2.6 cd	94	0	9.4 b	379.0 ab	2.52	-0.64
BE 5	2.6 cd	100	0	13.7 c	389.1 ab	1.15	-0.27*
SIC 806	3.0 de	100	0	9.3 b	532.9 bc	2.06	-0.48
CAS 2	3.3 ef	100	0	20.5 d	814.5 cd	2.06	-0.46*
SIAL 325	3.4 ef	100	7	6.4 ab	1780.3 e	1.98	-0.64
SIC 23	3.7 fg	100	27	9.6 b	992.5 d	1.41	-0.82
SIC 17	3.9 fg	100	27	7.2 ab	2953.7 f	1.36	-0.89

Table 4-3 continued.

Cultivar	Severity index[t]	Infection (%)[u]	Mortality (%)[v]	# Colonies/ root system[w]	# Propagules/ g soil[x]	Root wt.[y] (g)	Root wt. loss[z] (g)
SIC 19	4.1 gh	100	40	9.7 b	3774.2 g	1.15	-1.01
EET 228	4.3 h	100	40	15.0 b	943.3 d	1.65	-0.99

[t]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap roots; 5=plant moribund (wilted) or dead. Means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.05$); comparisons were valid within the same column.

[u]Infection is expressed as the percentage of 30 inoculated plants that were infected 8 weeks after inoculation as determined by plating roots on a selective medium.

[v]Mortality is expressed as the percentage of 30 inoculated plants that had died 8 weeks after inoculation.

[w]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.

[x]Average number of propagules recovered from 10 soil samples from each of 30 plants per cultivar 8 weeks after infestation (1 ml of a dilution of each sample was distributed on each of 10 plates of a selective medium).

[y]Average root weight of 30 plants.

[z]Difference between average root weights of the control and inoculated plants. Positive values represent greater root weights of infected plants than of control plants, while negative values indicate lower root weights of infected plants as compared to control plants. Values followed by an asterisk were not significantly different from the noninoculated control according to Dunnett's test ($P<0.05$).

Table 4-4. Disease severity, percentages of infection and mortality, colonies per root system, soil population, and root weight 8 weeks after planting 8-week-old cacao seedlings of 15 cultivars in soil infested with 5 x 10^5 zoospores of Phytophthora capsici per plant.

Cultivar	Severity index[t]	Infection (%)[u]	Mortality (%)[v]	# Colonies/ root system[w]	# Propagules/ g soil[x]	Root wt.[y] (g)	Root wt. loss[z] (g)
PA 30	0.6 a	60	0	1.1 a	0.0 a	2.96	-0.25*
PA 169	1.1 ab	80	0	3.3 ab	30.7 ab	3.00	-0.07*
PA 121	1.4 bc	80	0	3.9 bc	92.0 abc	2.91	-0.33*
SIC 806	1.5 bc	74	0	5.1 bc	112.3 abc	2.60	0.06*
EEG 8	1.6 bc	87	0	4.1 bc	163.7 cd	2.82	0.32*
CA 3	1.7 bc	87	0	5.6 c	61.5 abc	3.12	0.25*
EET 59	1.7 bc	97	0	5.3 bc	215.1 de	3.35	0.19*
BE 5	1.8 c	87	0	5.9 c	71.5 abc	2.87	0.03*
CAS 2	1.9 c	97	0	5.1 bc	232.7 de	3.27	0.74
EET 228	1.9 c	87	0	5.9 c	122.7 bcd	2.58	-0.07*
MA 15	2.5 d	100	0	8.5 d	91.9 abc	2.74	0.01*
SIAL 325	2.6 d	100	0	9.1 de	296.9 e	2.90	0.29*
SIC 17	2.7 de	100	0	10.8 e	429.3 f	2.03	-0.23*

Table 4-4 continued.

Cultivar	Severity index[t]	Infection (%)[u]	Mortality (%)[v]	# Colonies/ root system[w]	# Propagules/ g soil[x]	Root wt.[y] (g)	Root wt. loss[z] (g)
SIC 19	2.8 de	100	0	8.3 d	574.1 g	1.58	-0.57
SIC 23	3.3 e	100	0	10.4 de	1066.7 h	2.33	0.09*

[t]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap roots; 5=plant moribund (wilted) or dead. Means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.05$); comparisons were valid within the same column.

[u]Infection is expressed as the percentage of 30 inoculated plants that were infected 8 weeks after inoculation as determined by plating roots on a selective medium.

[v]Mortality is expressed as the percentage of 30 inoculated plants that had died 8 weeks after inoculation.

[w]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.

[x]Average number of propagules recovered from 10 soil samples from each of 30 plants per cultivar 8 weeks after infestation (1 ml of a dilution of each sample was distributed on each of 10 plates of a selective medium).

[y]Average root weight of 30 plants.

[z]Difference between average root weights of the control and inoculated plants. Positive values represent greater root weights of infected plants than of control plants, while negative values indicate lower root weights of infected plants as compared to control plants. Values followed by an asterisk were not significantly different from the noninoculated control according to Dunnett's test ($P<0.05$).

Table 4-5. Disease severity, percentages of infection and mortality, colonies per root system, soil population, and root weight 8 weeks after planting 8-week-old cacao seedlings of 15 cultivars in soil infested with 5 x 10^5 zoospores of Phytophthora megakarya per plant.

Cultivar	Severity index[t]	Infection (%)[u]	Mortality (%)[v]	# Colonies/ root system[w]	# Propagules/ g soil[x]	Root wt.[y] (g)	Root wt. loss[z] (g)
CAS 2	0.3 a	47	0	0.4 a	0.0 a	2.80	0.27*
PA 121	0.4 a	40	0	0.7 ab	0.0 a	3.33	0.80*
CA 3	0.4 a	47	0	0.9 abc	0.0 a	2.95	0.08*
PA 30	0.5 ab	47	0	1.1 abc	0.0 a	3.29	0.31*
PA 169	0.5 ab	53	0	1.1 abc	40.8 abc	2.90	-0.16*
SIC 806	0.5 ab	53	0	0.8 ab	30.6 abc	2.73	0.18*
EET 59	0.5 ab	53	0	1.2 abc	20.4 ab	3.14	-0.01*
EEG 8	0.5 ab	47	0	1.2 abc	0.0 a	2.82	0.31*
MA 15	0.7 abc	73	0	1.8 bcd	20.4 ab	2.78	0.05*
BE 5	0.7 abc	73	0	1.8 bcd	71.5 c	2.82	-0.02*
SIAL 325	0.7 abc	73	0	1.7 bcd	61.2 bc	2.67	0.05*
SIC 23	0.9 bcd	80	0	2.0 cde	51.7 bc	2.12	-0.11*
SIC 17	1.0 cd	73	0	3.1 ef	122.5 d	2.40	0.14*

Table 4-5 continued.

Cultivar	Severity index[t]	Infection (%)[u]	Mortality (%)[v]	# Colonies/ root system[w]	# Propagules/ g soil[x]	Root wt.[y] (g)	Root wt. loss[z] (g)
SIC 19	1.1 cd	87	0	2.5 def	20.4 ab	2.32	0.17*
EET 228	1.3 d	87	0	3.5 f	51.1 bc	2.55	-0.09*

[t]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap roots; 5=plant moribund (wilted) or dead. Means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.05$); comparisons were valid within the same column.

[u]Infection is expressed as the percentage of 30 inoculated plants that were infected 8 weeks after inoculation as determined by plating roots on a selective medium.

[v]Mortality is expressed as the percentage of 30 inoculated plants that had died 8 weeks after inoculation.

[w]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.

[x]Average number of propagules recovered from 10 soil samples from each of 30 plants per cultivar 8 weeks after infestation (1 ml of a dilution of each sample was distributed on each of 10 plates of a selective medium).

[y]Average root weight of 30 plants.

[z]Difference between average root weights of the control and inoculated plants. Positive values represent greater root weights of infected plants than of control plants, while negative values indicate lower root weights of infected plants as compared to control plants. Values followed by an asterisk were not significantly different from the noninoculated control according to Dunnett's test ($P<0.05$).

Table 4-6. Disease severity, percentages of infection and mortality, colonies per root system, soil population, and root weight 8 weeks after planting 8-week-old cacao seedlings of 15 cultivars in soil infested with 5 x 10^5 zoospores of Phytophthora heveae per plant.

Cultivar	Severity index[t]	Infection (%)[u]	Mortality (%)[v]	# Colonies/ root system[w]	# Propagules/ g soil[x]	Root wt.[y] (g)	Root wt. loss[z] (g)
EET 228	0.3 a	20	0	0.9 a	40.8 ab	2.65	0.00*
PA 30	0.5 ab	47	0	0.8 a	0.0 a	3.10	0.12*
PA 169	0.7 abc	53	0	1.7 ab	51.0 abc	3.01	-0.05*
PA 121	0.7 abc	67	0	1.4 ab	163.7 def	2.30	-0.25*
EET 59	0.7 abc	67	0	1.4 ab	0.0 a	3.48	0.32*
SIC 806	0.9 bcd	80	0	2.3 bc	163.5 def	2.62	0.08*
CA 3	0.9 bcd	80	0	1.8 ac	81.7 abcd	3.12	0.25*
EEG 8	1.0 cde	80	0	2.3 bc	132.8 cde	2.48	0.29*
BE 5	1.1 cde	87	0	2.7 bc	173.7 ef	2.74	-0.10*
MA 15	1.2 de	87	0	3.1 cd	122.5 bcd	2.81	0.08*
CAS 2	1.3 ef	87	0	4.1 de	235.3 fg	2.54	0.01*
SIAL 325	1.6 fg	100	0	4.9 ef	450.7 h	2.75	0.13*
SIC 23	1.6 fg	100	0	5.2 ef	522.6 h	2.32	0.09*

Table 4-6 continued.

Cultivar	Severity index[t]	Infection (%)[u]	Mortality (%)[v]	# Colonies/ root system[w]	# Propagules/ g soil[x]	Root wt.[y] (g)	Root wt. loss[z] (g)
SIC 19	1.8 g	100	0	5.4 ef	307.0 g	2.26	0.11*
SIC 17	2.0 g	100	0	5.7 f	502.1 h	2.24	-0.02*

[t]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap roots; 5=plant moribund (wilted) or dead. Means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.05$); comparisons were valid within the same column.

[u]Infection is expressed as the percentage of 15 inoculated plants that were infected 8 weeks after inoculation as determined by plating on a selective medium.

[v]Mortality is expressed as the percentage of 15 inoculated plants that had died 8 weeks after inoculation.

[w]Average of total number of colonies obtained per root system as determined by plating roots on a selective medium.

[x]Average number of propagules recovered from 10 soil samples from each of 15 plants per cultivar 8 weeks after infestation (1 ml of a dilution of each sample was distributed on each of 10 plates of a selective medium).

[y]Average root weight of 15 plants.

[z]Difference between average root weights of the control and inoculated plants. Positive values represent greater root weights of infected plants than of control plants, while negative values indicate lower root weights of infected plants as compared to control plants. Values followed by an asterisk were not significantly different from the noninoculated control according to Dunnett's test ($P<0.05$).

Table 4-7. Disease severity index, percentages of infection and mortality and root weight 8 weeks after planting 8-week-old cacao seedlings of eight cultivars in soil infested with 100 chlamydospores of Phytophthora citrophthora per gram of soil.

Cultivar	Severity index[v]	Infection[w] %	Mortality[x] %	Root weight[y] (g)	Root wt loss[z] (g)
PA 121	2.30 a	100	0	5.36	-0.93
PA 169	2.93 ab	93	6	3.99	-1.82
CA 3	2.93 ab	87	6	3.51	-0.90
EET 228	3.47 bc	100	0	3.40	-0.47
CAS 2	4.47 bc	93	13	3.79	-1.29
SIAL 325	3.60 bc	87	13	3.65	-0.83
SIC 17	3.60 bc	93	0	3.96	-0.92
SIC 19	4.00 c	100	20	3.04	-1.23

[v]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap roots; 5=plant moribund (wilted) or dead. Means followed by the same letter were not Significantly different according to Duncan's multiple range test ($P<0.05$); comparisons were valid within the same column.

[w]Infection is expressed as the percentage of 15 inoculated plants that were infected 8 weeks after inoculation as determined by plating roots on a selective medium.

[x]Mortality is expressed as the percentage of 15 inoculated plants that had died 8 weeks after inoculation.

[y]Average root weight of 15 plants.

[z]Difference between average root weights of the control and inoculated plants. Positive values represent higher root weights of infected plants than of control plants, while a negative value indicate lower root weights of infected plants as compared to control plants. Differences followed by an asterisk were not significantly different from the noninoculated control according to Dunnett's test ($P<0.05$).

Table 4-8. Disease severity index, percentages of infection and mortality, and root weight, 8 weeks after planting 8-week-old cacao seedlings of eight cultivars in soil infested with 100 chlamydospores of Phytophthora palmivora per gram of soil.

Cultivar	Severity index[y]	Infection[w] %	Mortality[x] %	Root weight[y] (g)	Root wt loss[z] (g)
PA 169	1.75 a	75	0	5.63	-0.18*
CA 3	1.85 a	85	0	3.97	-0.44*
PA 121	1.90 a	100	0	5.85	0.26*
CAS 2	2.30 a	95	0	4.71	-0.35*
EET 228	2.35 a	80	0	3.03	-0.84
SIAL 325	2.50 ab	80	0	3.65	-0.83
SIC 19	3.15 bc	85	0	3.95	0.32*
SIC 17	3.50 c	100	15	3.12	-1.75

[y]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap roots; 5=plant moribund (wilted) or dead. Means followed by the same letter were not significantly different according to Duncan'a multiple range test ($P<0.05$); comparisons were valid within the same column.

[w]Infection is expressed as the percentage of 20 inoculated plants that were infected 8 weeks after inoculation as determined by plating roots on a seletive medium.

[x]Mortality is expressed as the percentage of 20 inoculated plants that had died 8 weeks after inoculation.

[y]Average root weight of 20 plants.

[z]Difference between average root weights of the control and infected plants. Positive values represent higher root weights of infected plants than of control plants, while a negative value indicate lower root weights of infected plants as compared to control plants. Differences followed by an asterisk were not significantly different from the noninoculated control according to Dunnett's test ($P<0.05$).

Table 4-9. Disease severity index, percentages of infection and mortality, and root weight 8 weeks after planting 8-week-old cacao seedlings of eight cultivars in soil infested with 100 chlamidospores of Phytophthora megakarya per gram of soil.

Cultivar	Severity index[v]	Infection[w] %	Mortality[x] %	Root weight[y] (g)	Root wt loss[z] (g)
CA 3	0.17 a	22	0	4.44	0.03*
PA 121	0.17 a	17	0	6.61	0.32*
PA 169	0.28 a	17	0	5.69	0.12*
EET 228	0.22 a	22	0	3.91	-0.15*
SIAL 325	0.33 a	22	0	4.54	0.06*
SIC 17	0.56 a	33	0	4.34	-0.54
SIC 19	0.44 a	22	0	4.58	0.31*
CAS 2	0.50 a	33	0	5.72	0.66

[v]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap roots; 5=plant moribund (wilted) or dead. Means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.05$); comparisons were valid within the same column.

[w]Infection is expressed as the percentage of 18 inoculated plants that were infected 8 weeks after inoculation as determined by plating roots on a selective medium.

[x]Mortality is expressed as the percentage of 18 inoculated plants that had died 8 weeks after inoculation.

[y]Average root weight of 18 plants.

[z]Difference between average root weights of the control and infected plants. Positive values represent higher root weights of infected plants than of control plants, while a negative value indicate lower root weights of infected plants as compared to control plants. Differences followed by an asterisk were not significantly different from the noninoculated control according to Dunnett's test ($P<0.05$).

Table 4-10. Disease severity index, percentages of infection and mortality root weight, 8 weeks after planting 8-week-old cacao seedlings of eight cultivars in soil infested with 100 oospores of Phytophthora capsici per gram of soil.

Cultivar	Severity index[v]	Infection[w] (%)	Mortality[x] (%)	Root weight[y] (g)	Root wt loss[z] (g)
PA 169	0.0 a	0	0	5.75	-0.06*
PA 121	0.0 a	0	0	6.41	0.16*
CA 3	0.0 a	0	0	4.43	0.02*
CAS 2	0.0 a	0	0	5.42	0.36*
SIAL 325	0.0 a	0	0	4.49	0.01*
EET 228	1.4 b	71	29	3.48	-0.39*
SIC 17	1.7 bc	43	0	3.76	-1.12
SIC 19	2.5 c	71	29	3.45	-0.82

[v]Severity index: 0=no roots infected; 1=one to four first-order roots infected; 2=four to seven first-order roots infected; 3=one or two second-order roots infected or more than seven first-order roots infected; 4=three or more second-order roots infected, or one or more infections or cankers on tap roots; 5=plant moribund (wilted) or dead. Means followed by the same letter were not significantly different according to Duncan's multiple range test ($P<0.05$); comparisons were valid within the same column.

[w]Infection is expressed as the percentage of 13 inoculated plants that were infected 8 weeks after inoculation as determined by plating roots on selective medium.

[x]Mortality is expressed as the percentage of 13 inoculated plants that had died 8 weeks after inoculation.

[y]Average root weight of 13 plants.

[z]Difference between average root weights of the control and infected plants. Positive values represent higher root weights of infected plants than of control plants, while a negative value indicate lower root weights of infected plants as compared to control plants. Differences followed by an asterisk were not significantly different from the noninoculated control according to Dunnett's test ($P<0.05$).

Table 4-11. Average lesion areas and percentage of mortality 18 days after stem inoculations of 6-month-old cacao seedlings with discs of mycelium of Phytophthora spp.

Cultivar	P. citrophthora		P. palmivora		P. capsici		P. megakarya	
	Lesion area[y] (cm^2)	Mort.[z] (%)	Lesion area (cm^2)	Mort. (%)	Lesion area (cm^2)	Mort. (%)	Lesion area (cm^2)	Mort. (%)
PA 121	5.11 a	10	3.18 ab	0	2.50 a	0	0.38 a	0
PA 169	6.40 ab	10	3.23 ab	0	2.82 ab	0	0.44 a	0
CAS 2	7.20 abc	15	2.68 a	0	2.88 ab	0	0.45 a	0
SIC 19	7.93 bc	25	5.14 c	10	3.37 ab	0	0.58 b	0
CA 3	9.05 cd	10	4.41 bc	5	3.04 ab	0	0.42 a	0
SIAL 325	9.35 cde	20	3.89 abc	0	3.73 b	0	0.38 a	0
SIC 23	10.60 de	45	3.99 abc	0	3.34 ab	5	0.35 a	0
SIC 17	10.87 de	20	5.57 c	15	3.11 ab	0	0.38 a	0
EET 228	11.41 e	10	4.97 c	10	3.54 ab	0	0.41 a	0

[y]Mean lesion area for 20 plants of each cultivar. Means within a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P<0.05$).

[z]Mortality is expressed as the percentage of 20 inoculated plants that had died 18 days after inoculation.

in Table 4.6. The effects of species and cultivars, as well as the interaction of species with cultivars, were highly significant ($P<0.05$). The effect of each species over all of the cultivars was analyzed separately.

Phytophthora citrophthora and P. palmivora caused the highest levels of infection and disease severity in most of the cultivars inoculated with zoospores ($P<0.05$). Lower levels of infection occurred with P. heveae and P. megakarya than with the other three species. None of the cultivars inoculated with P. megakarya were 100% infected and only four cultivars, SIC 325, SIC 17, SIC 19, and SIC 23, showed 100% infection with P. heveae (Tables 4.5 and 4.6).

No immunity was observed in any of the cultivars tested with zoospore inoculum (Tables 4.2 to 4.6). Although infection levels were high in all cultivars, only 7 to 40% of seedlings of five of the cultivars died after inoculation with P. citrophthora or P. palmivora (Tables 4.2 and 4.3). Additionally, root weight losses did not occur after inoculation with P. heveae and P. megakarya; only SIC 19 had lower root weight in plants inoculated with P. capsici than in control plants; and only six of the cultivars inoculated with P. citrophthora and ten cultivars inoculated with P. palmivora had root weights less than those of the controls (Tables 4.2, 4.3, 4.4, 4.5, and 4.6). Thus, high infection rates generally did not result in high incidences of disease or mortality, and the cultivars evaluated all had some level

of tolerance to root infection by each of the five species of Phytophthora.

Based on the disease severity index, root weight loss, and mortality, cultivars BE 5, EEG 8, PA 30, and PA 121 were highly tolerant to all species of Phytophthora. None of the Phytophthora spp. caused significant root weight losses or mortality in these cultivars. All of the cultivars tested except SIC 19, which had lower root weight in plants inoculated with P. capsici than in the controls, were highly tolerant to P. capsici, P. heveae, and P. megakarya. Based on high disease severity index values, 7 to 40 per cent mortality, and significant root weight losses in inoculated plants, SIC 17, SIC 19, SIC 23, EET 228, and SIAL 325 all had low tolerance to P. citrophthora and P. palmivora (Tables 4.2 and 4.3). These five cultivars also generally had the highest disease severity indices with P. capsici, P. heveae, and P. megakarya (Tables 4.4 to 4.6). Cultivars CA 3, EET 59, MA 15, PA 169 and SIC 806 had disease severity indices of 3.0 or less, but significantly lower root weights were observed in plants of these cultivars inoculated with P. citrophthora or P. palmivora than in control plants; they, along with CAS 2, appeared to be of intermediate tolerance to one or both of the latter two fungi (Tables 4.2 and 4.3).

The highest numbers of colonies of all of the Phytophthora spp. per root system were generally observed

with cultivars that had the highest severity indices, but some exceptions occurred (Tables 4.2 to 4.6). Plants of cultivars SIC 19 and SIC 23, when infected with P. citrophthora (Table 4.2), had relatively fewer colonies growing from their root systems than did other cultivars that were considered to have low tolerance; however, the colonies from SIC 19 and SIC 23 originated from infections on tap roots, which contributed to a high disease severity index. Conversely, cultivar CAS 2 had more colonies per root system than any other cultivar inoculated with P. palmivora, but no root weight loss occurred and it had a lower disease severity index because the infections were restricted to first order roots (Table 4.3). In general, P. citrophthora and P. palmivora formed more colonies on root systems of all the cultivars than did the other Phytophthora spp.

High numbers of propagules of P. citrophthora and P. palmivora were detected in soil from most of the cultivars. Populations were consistently highest in soil from the least tolerant cultivars, such as SIC 17, SIC 19, and SIC 23. The cultivars CA 3, PA 30, PA 121, PA 169, and EET 59 were always among the cultivars from which low numbers of propagules of the five Phytophthora spp. were recovered. No propagules were detected in samples of soil from around the root systems of plants of five cultivars infected with P. megakarya, PA 30 or EET 59 infected with P. heveae, or PA 30

infected with P. capsici or P. citrophthora. Low levels of propagules of Phytophthora spp. were noted also in soil from several other cultivars. For example, low levels of P. citrophthora and P. capsici were detected in soil from plants of the cultivar BE 5.

When eight cultivars were planted in soil infested with 100 chlamydospores per gram of soil, higher levels of infection occurred with P. citrophthora and P. palmivora than with P. megakarya (Tables 4.7 to 4.9). Only 16-33% infection of plants and no mortality were observed with P. megakarya (Table 4.9). Only SIC 17 had significantly lower root weight after growth in soil infested with chlamydospores of P. megakarya as compared to controls in non-infested soil. Plants of CAS 2 growing in soil infested with this species, however, had greater root weights than control plants. Thus, all eight cultivars were highly tolerant to P. megakarya at the level of inoculum tested.

Based on low disease severity indices and no significant root weight loss, CA 3, PA 121, PA 169, and CAS 2 were highly tolerant to P. palmivora. The high disease severity, severe root weight loss and 15% mortality of SIC 17 indicated that it has only low tolerance to P. palmivora. Based on high disease severity indices, reduced root weights, and 6 to 20% mortality in five of the eight cultivars grown in soil infested with chlamydospores, all cultivars tested had only low tolerance to P. citrophthora. This experiment was

performed early in the course of the studies, and no data were collected for total number of colonies growing from the root systems of the cultivars or for soil populations of the fungi.

When seedlings of the same eight cultivars were planted in soil infested with 100 oospores of P. capsici per gram of soil, only roots of the cultivars EET 228, SIC 17 and SIC 19 were infected (Table 4.10). Root weights of SIC 17 and SIC 19 were lower than those of the control plants, and mortality was observed in 28% of the seedlings of the cultivars EET 228 and SIC 19. Thus, all of the cultivars except EET 228, SIC 17 and SIC 19 were highly tolerant when grown in soil infested with oospores of P. capsici.

Lesions were formed on the stems of all seedlings of the eight cultivars tested 18 days after inoculation with each of the five species of Phytophthora (Table 4.11). Phytophthora citrophthora caused the largest lesions in all cultivars. Mortality of some plants in all cultivars was observed with this species; 45% of the inoculated seedlings of SIC 23 died. Very small lesions and no plant mortality were observed with P. megakarya, and, as occurred before with chlamydospore or zoospore inoculum, all cultivars showed tolerance to this species. Lesions caused by P. megakarya in inoculated seedlings of SIC 19 were slightly, but significantly, greater than those observed on other cultivars. Stem lesions of sizes intermediate to those

caused by P. citrophthora and P. megakarya occurred after inoculation with P. capsici and P. palmivora. Little or no mortality occurred after inoculation with P. capsici, and only 5 to 15% mortality was observed with seedlings of CA 3, SIC 19, SIC 17, and EET 228 inoculated with P. palmivora. Seedlings of the cultivar EET 228 did not develop as well as the other cultivars and consequently they were less tolerant to stem inoculation. When inoculated with P. citrophthora and P. palmivora, PA 121, PA 169, and CAS 2 had smaller lesions than SIC 17, SIC 23 and EET 228, and thus were more tolerant. The average lesion area caused by P. citrophthora on cultivar SIC 19 was smaller than lesions caused in other cultivars that are considered to have low tolerance, but it was a consequence of wilting and death of five of the inoculated plants for which lesion area could not be evaluated. Consequently, those values were not analyzed and the lesion areas of this cultivar were underestimated.

Discussion

Phytophthora citrophthora and P. palmivora caused more damage than P. capsici, P. heveae, or P. megakarya to cacao seedlings in all of the tests with chlamydospores or zoospores in root inoculations, and also with stem inoculations. Phytophthora megakarya was consistently the least virulent of the five Phytophthora spp. tested in both stem

lesion assays and root inoculations of cacao seedlings. These results are consistent with and support the results discussed in Chapters 2 and 3. Lawrence and Luz (80) and Campelo et al. (28) also found that P. citrophthora and P. palmivora caused more damage than P. capsici in pods, stems, and germinated seeds of cacao.

Although unnaturally high levels of inoculum were used and roots were readily infected, little mortality or root weight loss occurred with most of the cultivars in this study. The cultivars evaluated, thus, are termed tolerant, rather than resistant, because they were infected but generally sustained little or no apparent damage in terms of mortality, root weight loss, yellowing, or loss of vigor. The 15 cultivars tested by root inoculations and the nine cultivars tested by stem inoculations demonstrated different levels of tolerance to the five Phytophthora spp. over the eight weeks of the tests, but immunity was not observed. Low tolerance to root disease was observed on five (EET 228, SIC 17, SIAL 325, SIC 23 and SIC 19) of the 15 cultivars inoculated with P. citrophthora or P. palmivora, which killed up to 40% of the plants of these cultivars. As has been observed before in Brazil with pod inoculations, (80; Luz et al. unpublished) high levels of tolerance or resistance will be more difficult to find for P. citrophthora and perhaps P. palmivora than for the other Phytophthora spp.

The cultivars PA 30, PA 121, PA 169, CA 3, CAS 2, SIC 806, and MA 15 have been reported to have high levels of resistance to P. capsici, P. citrophthora, and P. palmivora in screening tests using attached pod inoculations (59, 88, 122, 124, 129, Luz et al. unpublished). High levels of resistance have been reported for MA 15, BE 5, and EEG 8 in tests with stem inoculations (114). Cultivars PA 30, PA 121, EEG 8, and BE 5 were also evaluated in this study and found to be highly tolerant to root disease when inoculated with zoospores of all five species of Phytophthora. Cultivars CAS 2 and MA 15 were highly tolerant to all Phytophthora spp. except P. palmivora; because CAS 2 had a disease severity value of 3.3 with P. palmivora, which was significantly higher than the values for the highly tolerant cultivars with P. palmivora, and MA 15 had significant root weight loss, both were considered to have intermediate tolerance to this pathogen. Although cultivars CA 3, PA 169, and SIC 806 had disease severity indices that were not significantly greater than those for several other highly tolerant species, root weight losses occurred with both P. citrophthora and P. palmivora and these cultivars were considered to have intermediate tolerance to root disease caused by the latter two pathogens. On the same basis, EET 59 was considered to have intermediate tolerance to P. palmivora. Of the cultivars reported above to be resistant or tolerant based on pod or stem inoculations, only PA 121,

PA 169, CA 3, and CAS 2 were included in tests with chlamydospore and oospore inoculum. All of these cultivars were highly tolerant when grown in soil infested with chlamydospores or oospores of P. capsici, P. megakarya, and P. palmivora; however, they all had low or intermediate tolerance to P. citrophthora. The low levels of tolerance of seedlings of the cultivars SIC 17, SIC 19, SIC 23, and SIAL 325 to root infection with P. citrophthora and P. palmivora support previous reports on the susceptibility of these cultivars to pod inoculations with P. citrophthora and P. palmivora in Brazil (80, 88, 129; Luz et al. unpublished). The rankings of cultivars PA 121 and SIC 19 were similar to those reported in Chapters 2 and 3.

Generally the levels of tolerance detected with root inoculations were similar to those observed for stem inoculations in this study. For example, in both tests cultivars PA 121, PA 169 and CAS 2 were more tolerant than SIC 17 and EET 228 to P. citrophthora and P. palmivora. However, relative results for the same cultivar were not always consistent with the different inoculation methods. For example, SIC 23 had low tolerance to P. palmivora based on tests with zoospores, but, based on stem inoculations, it was highly tolerant.

The disease severity index employed in these studies has been demonstrated to be a reliable parameter for the comparison of levels of infection and tolerance among cacao

cultivars to root infection by Phytophthora spp. The usefulness of this index supports previous observations in Chapter 3 of this dissertation and the findings of Frosheiser and Barnes (52) and Basu (13) for evaluations of resistance to Phytophthora root rot in alfalfa plants. The disease severity index allowed more critical differentiation of tolerance among cultivars than did the other individual quantitative parameters used in this study. Root weights of plants, for example, were extremely variable. Root weight is associated with the plant phenology and directly expresses the genetic variability existing among progenies of each cultivar. The use of total dry weight of plants to evaluate responses of cacao seedlings and clones to infection of roots by P. palmivora may have been at least partially responsible for the failure of several workers to obtain good correlations among cultivars tested for root infection (10, 11, 76, 146, 149) and those reported to have tolerance or resistance based on pod infection or stem inoculations (76). However, for critical differentiations of tolerance based on a disease severity index, relative root weight loss and mortality should also be considered.

Root inoculations for the evaluation of resistance of cacao cultivars to P. palmivora have been used before with success in Ghana (8, 10, 11, 146, 149, 150) and in the U.S.A. (165). According to Zentmyer et al. (165), significant differences in resistance of various cacao clones to

stem inoculations and root inoculations occurred with P. palmivora. After Turner and Asomaning (146) suggested that resistance or tolerance was a general property of the plant tissue and not inherent only to pods, Asomaning (10) and Amponsah and Asare-Nyako (8) proposed that root inoculations might be of practical value in evaluating varieties for resistance to P. palmivora. However, Lawrence (76), in a comparative evaluation of all of the methods used to assess resistance of cacao cultivars to P. palmivora, stated that inoculations of seedling roots gave poor and inconsistent results as a method for screening hybrid progenies. The technique used by Lawrence (76), which was based on the method described by Turner and Asomaning (146), consisted of pouring zoospore suspensions on the soil surface at specified distances from the base of the stem. As indicated by Mitchell and Kannwischer-Mitchell (103), this technique does not allow optimal zoospore dispersal or inoculum efficiency for infection by Phytophthora spp. Inoculum in the present study was maintained under conditions favorable for zoospore dispersal to roots by flooding roots prior to inoculation, adding the inoculum suspension to the surface water, and maintaining the soil under flooded conditions before draining the infested water past roots (102). Root inoculations with zoospores in this study allowed the determination of levels of tolerance in cultivars and differences in virulence of the five Phytophthora spp., but support Lawrance's

(76) conclusion that roots sustain less disease disease than pods.

Root inoculations with 5 x 10^5 zoospores per plant or 100 chlamydospores per gram of soil resulted in differential responses of cultivars to infection by P. citrophthora and P. palmivora. Infection with zoospores of all species of Phytophthora tested occurred predominantly in the upper tap root and first- or second-order roots whereas infections from chlamydospores occurred in first- and second-order roots of both upper and lower parts of the root system. It is difficult to compare tests with zoospores and chlamydospores because the plants were grown under different conditions for the two inoculation methods and the two methods for inoculation were quite different. However, although similar trends in infection were observed among cultivars inoculated with either method, several differences occurred in loss of root weight and mortality. All cultivars grown in soil infested with chlamydospores of P. citrophthora had lower root weights than control plants, but differential losses in root weights among cultivars occurred with zoospore inoculum. Mortality was greater in cultivars of low tolerance when inoculated with zoospores than when exposed to chlamydospores. Different results for the two inoculation methods, however, may have been due to inoculum impact on plant root systems, which were larger in tests with chlamydospores than in tests with zoospores. Although either

spore form is satisfactory for root inoculations, zoospores are recommended for future screening programs because they appear to provide a more definitive assessment of tolerance and they can be produced more easily and quickly than chlamydospores. Usually a few carrot-agar cultures, if incubated under adequate conditions, produce enough sporangia and zoospores to inoculate many plants.

Additional studies need to be conducted with oospores of P. capsici to determine if the lack of infection in some cultivars that had high levels of infection with zoospores was due to differential inhibition of oospore germination by cultivars. Because of the difficult and time-consuming methodology required for oospore production, it does not appear that they would provide the basis for an efficient technique for screening for resistance or tolerance in cacao cultivars to Phytophthora spp., although their use may yield valuable information on the ecology and epidemiology of the diseases they cause.

Although the method of screening cultivars for root disease caused by Phytophthora spp. used in this study proved to be efficient for the evaluation of cultivars for tolerance, additional refinements are needed. The use of younger seedlings (less than 5 to 8 weeks old) or clonal root cuttings should be tested to simplify evaluation of disease in the root system. Young seedlings have less developed root systems and consequently disease may be more

easily and accurately assessed. Lower zoospore concentrations of P. citrophthora and P. palmivora may be required to differentiate disease among cultivars if young seedlings are used. The use of rooted cuttings should eliminate much of the intra-cultivar variability in disease reaction; variability in size and vigor of the open pollinated cultivars used in this study may have compromised the reliability of results with some cultivars, such as EET 228, because weak plants in the seed lot were less tolerant to infection, root disease, and stem cankers than other more vigorous plants in the same lot. Progenies of the same male parental descendance could also be used to diminish genetic variation, since, due to self-incompatibility in many cultivars, selfed progenies are not available (157, 158).

The differences in the soil populations of Phytophthora spp. associated with root infection in different cultivars suggested that tolerant or resistant cultivars may limit numbers of propagules formed in soil around infected roots. The association of resistance to root disease caused by Phytophthora spp. and soil populations of the pathogen has been studied in avocado (67) and citrus (2, 99). In both cases lower populations of the pathogens were associated with some resistant rootstocks more than with susceptible rootstocks, but other rootstocks thought to be resistant supported large populations of the pathogen. Kellam and Coffey (67) stated that the observed differences in the soil

populations of P. cinnamomi and root infection in two resistant avocado rootstocks may suggest that separate types of resistance mechanisms might operate in those two cultivars. According to Alt (5), who studied resistance to P. cactorum in apple cultivars, resistance of cultivars or plant species may in part be determined by the restriction of sporulation by the pathogen in infected host tissue. The low populations of Phytophthora spp. that developed with the cacao cultivar PA 30 and several other resistant cultivars in the present study suggested that they might restrict sporulation of Phytophthora spp. New root growth from infected roots was observed in some of the most tolerant cultivars, such as PA 30 and PA 121, but not in the least tolerant cultivars, such as EET 228 and SIC 19, (data not presented), and may also play an important role in tolerance or resistance to the disease. Differences in root regeneration between tolerant and susceptible roots have also been reported for several plants infected with P. cinnamomi (23, 57). The influence of roots of cacao cultivars with different levels of tolerance on soil populations of Phytophthora spp. should be evaluated further. If the ability of these cultivars to restrict the development of soil populations of Phytophthora spp. is confirmed, their use in breeding programs should be epidemiologically important in the control of black pod disease of cacao in the field because propagules produced in soil serve as inoculum for additional

root infections and for the onset of aerial epidemics of black pod disease in cacao plantations (54).

Chapter 5
SUMMARY AND CONCLUSIONS

Phytophthora palmivora, P. capsici, P. megakarya, and P. citrophthora have been reported to cause severe disease losses in cacao (20, 25, 28, 80). These species and at least two other species, P. megasperma and P. heveae, occur individually or in various combinations in different areas of the world where cacao is grown. Only by the determination of the capacities of the various Phytophthora spp. to cause disease on different parts of the cacao tree will the epidemiology of these diseases be clarified. The objectives of the research presented in this dissertation were to compare the roles of five Phytophthora spp. in diseases of pods, leaves, roots, and stems of cacao; to relate the effect of inoculum densities of Phytophthora spp. in soil to incidence of disease in cacao roots; and to evaluate selected cultivars for tolerance to root disease caused by Phytophthora spp.

Phytophthora capsici, P. citrophthora, P. heveae, P. megakarya, and P. palmivora were all pathogenic to detached pods, seedling stems, excised roots, and leaves of cacao trees, but great differences occurred in the degrees of virulence of the five species in relation to the different

parts of the cacao plant tested. Generally P. citrophthora was the most virulent species on all of the cacao parts tested. Phytophthora palmivora was also highly virulent on all parts of the cacao tree, while P. capsici was generally equally pathogenic as P. citrophthora and P. palmivora only on leaves. Lesions caused by P. heveae on pods, leaves, and excised roots were not different from the lesions caused by P. capsici and both of these species can be considered as moderate pathogens of cacao. Phytophthora megakarya was the least virulent among the five species of Phytophthora under the conditions of these tests.

Phytophthora spp. appear to have varied specialties that influence their survival and abilities to cause disease under different conditions. Phytophthora citrophthora is a severe threat to cacao plantations where it occurs because of its ability to cause disease on roots and trunks of cacao and associated plants, especially under flooded conditions (28, 70, 80; Luz and Mitchell, unpublished). Phytophthora palmivora, which is widely disseminated and has a wide variety of hosts (31) as compared to the other four species, has been isolated from naturally infected, pods, chupons, flowers, flower cushions, seedlings, trunks, and roots of cacao; this ability to infect many parts of the tree may lead to an abundance of inoculum that results in rapid spread in a cacao plantation if adequate environmental conditions occur. The natural adaptability of P. heveae to

infest soil and infect roots indicates a potential of this species to cause root disease in cacao plantations. Phytophthora capsici does not appear to be well adapted to soil and roots, but its ability of sporulate abundantly on the surfaces of infected aerial parts allows its survival and rapid spread inside the cacao canopy under favorable environmental conditions (85). Although P. megakarya was a poor pathogen compared to the other Phytophthora spp. under the present experimental conditions, this species may behave differently in its natural habit in West African countries. The role of this species in causing disease on different parts of cacao plants needs further evaluation with cultivars grown in Africa and in an area where most of the Phytophthora spp. occur naturally. Cameroon probably would be the best place to perform such tests.

The inoculation of excised sections of roots of cacao proved to be a potentially important method to test pathogenicity of Phytophthora spp. isolated from soil and roots in the type of host tissue in which they normally live, but under controlled and reproducible conditions. However, the bacterial contamination must be eliminated and the technique must be tested further if it is to be useful.

Zoospores, chlamydospores and oospores of Phytophthora spp. were able to infect roots of cacao seedlings and cause disease. Only the use of fragments of mycelium of P.

capsici did not result in infection in these experiments. All five Phytophthora spp. infected roots of 5- to 8-week-old cacao seedlings of five cultivars, and disease was most severe at the highest inoculum levels tested. Mortality generally was restricted to inoculations with P. citrophthora and P. palmivora at 10^6 zoospores per plant or 500 chlamydospores per gram of soil. As in the previous experiments, P. citrophthora and P. palmivora caused more damage to root systems of cacao seedlings than the other species did, and P. megakarya caused little damage and no mortality even at the highest concentration of zoospores or chlamydospores used. Mortality of plants exposed to zoospores or oospores of P. capsici occurred at low percentages and was inconsistent over repeated experiments. Two hundred and fifty oospores of P. heveae per gram of soil caused 50 and 80% mortality of plants of SIC 19 under non-flooded and flooded conditions, respectively, and caused more severe damage at this level of inoculum than P. capsici did.

All five Phytophthora spp. have the potential to infect cacao root systems, and, although the plants may not show external symptoms, the colonized roots may function as reservoirs of inoculum that may have subsequent roles in disease of the aerial parts of the cacao plant. Additionally, root infection and subsequent disease caused by Phytophthora spp. may contribute to increased severity of dieback that has been recorded in nearly every major cacao

producing country under drought conditions. The present results reinforce the hypothesis that root infection plays a major role in the epidemiology of cacao black pod disease (53, 54).

Flooding of infested soil has been reported to have a profound effect on disease severity caused by several Phytophthora spp. (15, 19, 93, 94, 154). However, experiments conducted with cacao seedlings demonstrate that flooding had little effect on disease caused by zoospores or chlamydospores of P. citrophthora, P. megakarya, and P. palmivora. In contrast, inoculation with oospores of P. capsici or P. heveae resulted in more severe damage to periodically flooded plants than to plants growing under freely drained conditions. Further studies need to be conducted with cacao grown under controlled water potentials with periodic flooding after inoculations with Phytophthora spp. to determine if disease incidence and inoculum efficiency are increased with flooding.

Considerable variations in disease responses occurred among replications of individual treatments and between experiments to determine the relationship between inoculum densities and disease incidence. Among the factors that may have been partially responsible for these variations were: i) the heterogenicity observed in cacao; ii) the use of seedlings from open pollinated progenies; iii) the variation in material obtained from different areas; and iv) the age

of the seedlings. However, in general, similar responses were obtained in experiments conducted in Florida and Brazil, and the cultivars had similar reactions to each species in spite of the different environmental conditions.

The use of resistant or tolerant cultivars is often one of the most effective and economical means of controlling plant diseases, and the search for cacao cultivars resistant or tolerant to black pod has been intensive in several parts of the world (8, 29, 43, 77, 116, 121). Because of the epidemiological significance discussed above, it is of equal importance to determine if cultivars that are resistant, tolerant, or susceptible to black pod respond similarly to root disease caused by each species of Phytophthora. Fifteen cacao cultivars, BE 5, CA 3, CAS 2, EEG 8, EET 228, EET 59, MA 15, PA 30, PA 121, PA 169, SIAL 325, SIC 17, SIC 19, SIC 23, and SIC 806, all previously tested for resistance to black pod (59, 79, 88, 116, 122, 129; Luz et al., unpublished), were inoculated with zoospores of Phytophthora spp. Although high levels of inoculum were used and roots were readily infected, little root weight loss or mortality occurred with most of the cultivars in this study. The cultivars demonstrated different levels of tolerance to the five Phytophthora spp., but cultivars PA 30, PA 121, EEG 8, and BE 5 were considered to be highly tolerant to root disease caused by all five Phytophthora spp. These cultivars all had high levels of infection of root systems;

however, they had low disease severity index values, which indicated that generally only first-order roots were infected, and they sustained no root weight loss or mortality of plants. The cultivars CA 3, PA 169, SIC 802, CAS 2, EET 59, and MA 15 were highly tolerant to P. capsici, P. heveae, and P. megakarya, but were considered of intermediate tolerance to P. citrophthora, P. palmivora, or both. With the latter two species, these cultivars had either high disease severity index values or sustained some root weight loss or mortality. Cultivars PA 121, PA 169, or CAS 2 were more tolerant than CA 3, EET 228, SIAL 325, SIC 17, SIC 19, and SIC 23 to stem inoculations with P. capsici, P. citrophthora, P. palmivora, and P. megakarya. Generally, the levels of tolerance detected with root inoculation were similar to those observed for stem inoculations in this study. However, dissimilarities between the inoculation methods also occurred.

The disease severity index employed in these studies was an important indicator of infection and disease, and it allowed comparisons of levels of tolerance among cacao cultivars to root disease caused by Phytophthora spp. The disease severity index incorporated values of number of colonies per type of root in the root system, disease incidence, and mortality. The location of the sites of infection is very important for the assessment of disease severity caused by Phytophthora spp. on cacao roots, and the

disease severity index quantitatively incorporates location of infected sites. However, for critical differentiation of tolerance or resistance, other disease parameters, such as root weight loss, should also be considered.

The differences in the soil populations of Phytophthora spp. associated with root infection in different cultivars suggested that tolerant or resistant cultivars may limit the number of propagules formed in soil around infected roots. If the ability of cultivars such as PA 30 and PA 121 to restrict the development of populations of Phytophthora spp. is confirmed, their use in breeding programs should be epidemiologically important in the control of black pod disease.

APPENDIX

Table A-1. Percentages of germination of zoospores of Phytophthora spp. on water-agar 24 hours after plating zoospore suspensions used for soil infestation in experiments conducted at CEPLAC and at the University of Florida.

Table	Exp. #	% Germination[y]				
		P. citrophtora	P. palmivora	P. capsici	P. megakarya	P. heveae
3.2-3.3	1+2	90	94	95	80	-[z]
3.8-3.9	1	85	90	92	85	-
3.8-3.9	2	88	94	94	85	89
3.10-3.11	1	84	93	97	82	-
3.10-3.11	2	90	90	92	83	-
4.2-4.6	1	88	95	96	85	92
4.2-4.6	2	90	94	94	83	-

[y]Expressed as the percentage of 100 zoospores per sample that produced a germ tube of 5 microns or more in length.
[z]"-" = Species not tested in this experiment.

Table A-2. Populations of Phytophthora spp. detected in soil 24 hours after soil infestation with chlamydospores.

Table	Experiment #	Calculated ID	Propagules per gram of soil[y]		
			P. citrophthora	P. palmivora	P. megakarya
3.4-3.5	1+2	100	94	93	156[z]
3.12-3.13	1	100	92	96	76
		250	233	230	188
3.12-3.13	2	100	97	95	78
		250	238	235	200
3.14-3.15	1	100	96	98	78
		250	235	230	180
		500	480	470	380
3.14-3.15	2	100	93	96	74
		250	238	235	190
		500	460	480	400
4.7-4.9	1	100	95	96	78
	2	100	94	94	76

Table A-2. Continued.

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[y]Number of colonies detected from each sample of soil 48 hours after plating 1.0 ml of a 10^{-1} or 10^{-2} dilution on each of 10 plates of a medium selective for *Phytophthora* spp.

[z]In this experiment *P. megakarya* was added to soil as 200 chlamydospores per gram of soil.

Table A-3. Percentage of germination of oospores of Phytophthora heveae in soil dilution samples 24 hours after soil infestation.

Table	Experiment	ID	% Germination[z]
3.6-3.7	1+2	100	27
		250	34
3.16-3.17	1	100	32
		250	28
3.16-3.17	2	100	34
		250	28

[z]Expressed as percentage of propagules giving rise to colonies detected on a medium selective for Phytophthora spp. 72 hours after plating a 1.0-ml sample of a 10^{-1} dilution of infested soil on each of 10 plates.

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BIOGRAPHICAL SKETCH

Edna Dora Martins Newman Luz was born in Recife, Pernambuco, Brazil. She received a degree of Engineer Agronomist in December, 1972, from the Universidade Federal Rural de Pernambuco, Recife, Brazil. From 1973 to 1974 she served as a researcher at the Instituto de Pesquisa Agropecuaria do Nordeste (IPEANE) in Recife. From 1974 to 1979, she served as researcher at the Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA). She received a degree of Master of Science in plant pathology from the Universidade Federal de Vicosa, Minas Gerais, Brazil in May, 1977. From 1979 until now, she has been serving as a researcher in the plant pathology division at Comissao Executiva do Plano da Lavoura Cacaueira (CEPLAC). In December, 1985, she received an award from CEPLAC, offered annually to the best researcher of the Cocoa Research Center at CEPLAC. Edna and Emanoel Acilino Teotonio da Luz were married in Recife, Brazil, in January, 1975. She began her studies in January, 1986 towards the degree of Doctor of Philosophy in the field of plant pathology at the University of Florida.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. David J. Mitchell, Chairman
Professor of Plant Pathology

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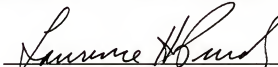

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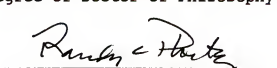

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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